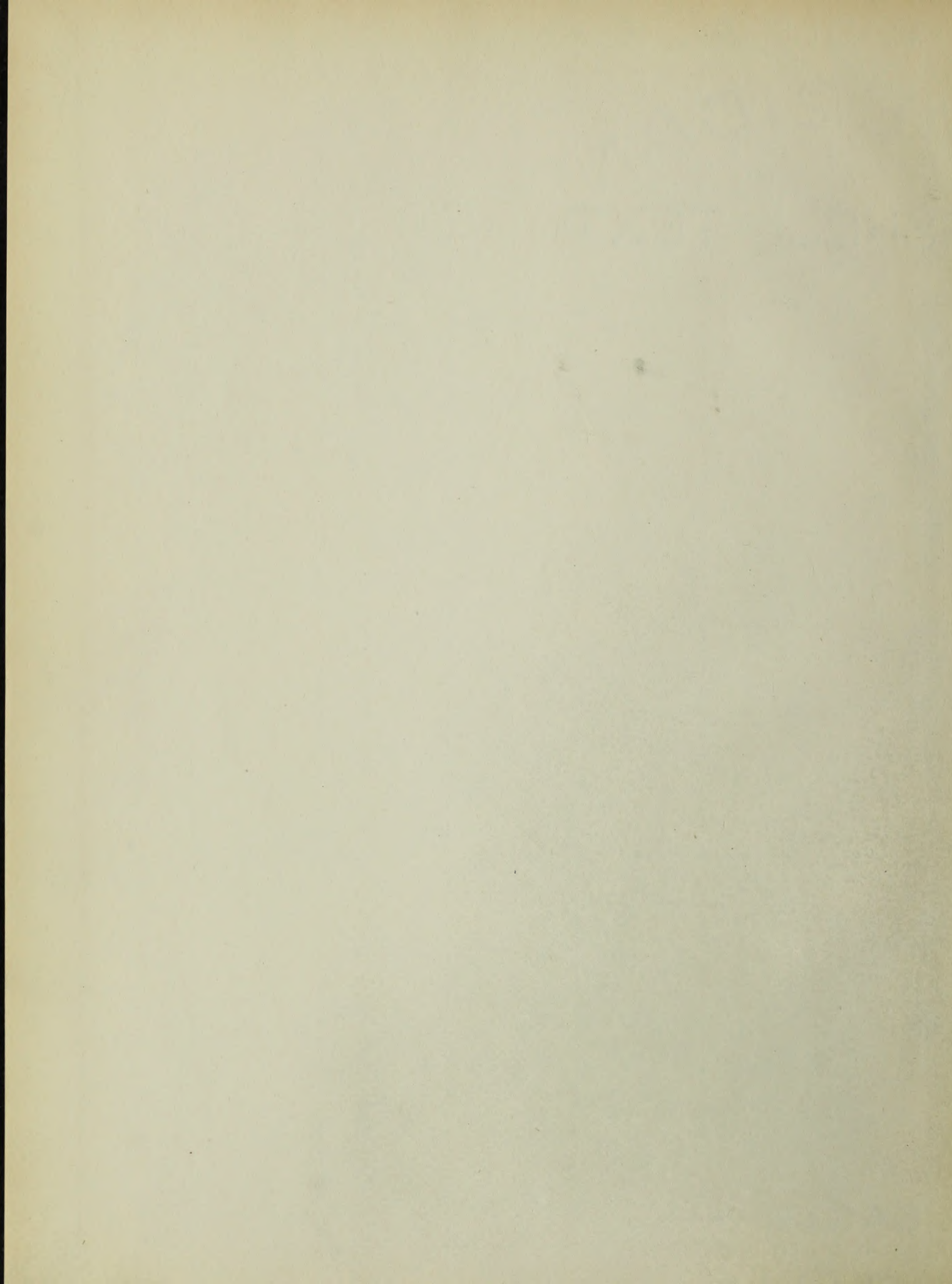




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1962

BOSTON UNIVERSITY

GRADUATE SCHOOL

Dissertation

CARBOHYDRATE METABOLISM IN RATS WITH ADRENOCORTICAL TRANSPLANTS

by

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(S.B., Massachusetts Institute of Technology, 1926;

A.M., Boston University, 1941)

Submitted in partial fulfilment of the

requirements for the degree of

Doctor of Philosophy

1943

PhD

1943

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Approved

by

Richard C. Wyman

Professor of Physiology

Charles J. W. W.

Professor of Biochemistry

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GENESIS OF THIS THESIS

The suggestion which led to this research was made by Dr. Leland C. Wyman, Professor of Physiology at the Boston University School of Medicine.

He cited the observation made by Russell¹ that

"the problem of carbohydrate metabolism in adrenalectomized rats is in some confusion owing . . . to the frequent use of animals in the terminal stages of adrenal insufficiency, when physiological changes, only indirectly the results of adrenalectomy, may influence the results."

He further pointed out that he and tum Suden² had shown that

"Transplanted adrenal cortical tissue, or that regenerated from accessories or operative rests, although capable of maintaining life, general health and resistance to stress, is not able to function as efficiently as does the normal gland in acute emergencies"

It seemed therefore, that by carrying out a study of the carbohydrate metabolism in the rat with transplanted adrenal cortical tissues, some of the disadvantages mentioned by Russell would be eliminated, and information might be gained which would be of considerable value.

.....

1. Russell, J.A. Am. J. Physiol. 128: 552. 1940

2. Wyman, L.C. and C. tum Suden. Endocrinology. 21: 587. 1937

INTRODUCTION

ACKNOWLEDGEMENT

I wish to acknowledge the many valuable suggestions given me by Dr. Leland C. Wyman in carrying out this investigation.

Grateful thanks are due also to Dr. Caroline tum Suden for her generous counsel, and for performing the surgical procedures.

Carbohydrate metabolism is most affected by adrenal insufficiency. The absorption is reduced. This reduction coupled with a possible increased utilization, may account for the flattened glucose tolerance curve found in patients with Addison's disease. The ability to form carbohydrates from non-carbohydrate sources (such as protein) is diminished or abolished. In the other hand, large doses of adrenal extract . . . increases the liver glycogen considerably above the normal level. The decrease of fasting blood sugar may be accounted for by increased utilization and diminished glycogenesis.

It is to be noted that much of these conclusions is based on results obtained from observations made on animals which are of considerable difficulty within a comparatively short time after operation.

J. Harrison, M.D., J. Amer. Med. Assoc., 17: 1403, 1921

INTRODUCTION

It is a well established fact that the chief carbohydrates present in the body consist of the 'sugar' in the blood and tissue fluids, and the glycogen stored in the tissues, principally in the liver and muscles. These substances exist in varying amounts dependent on a large number of factors, such as the amount of carbohydrate ingested, the needs of the organism for carbohydrate, the action of the nervous system, and, in particular, the modifying effects of the secretions elaborated by the endocrine glands. That the cortex of the adrenal gland exerts such effects is now well known. In a recent paper, Hartman³ has said of this relationship:

"Carbohydrate metabolism is most affected by adrenal insufficiency. The absorption is reduced. This reduction coupled with a possible increased utilization, may account for the flattened dextrose tolerance curve found in patients with Addison's disease. The ability to form carbohydrate from non-carbohydrate sources (such as protein) is diminished or abolished. On the other hand, large doses of adrenal extract . . . can raise the liver glycogen considerably above the normal level. The decreased fasting blood sugar may be accounted for by increased utilization and diminished glyconeogenesis."

It is to be noted that much of these conclusions is based on results obtained from observations made on animals which die of acute adrenal insufficiency within a comparatively short time after operation.

.....

3. Hartman, F.A. J. Amer. Med. Assoc. 117: 1405. 1941

INTRODUCTION

It is a well established fact that the chief carbohydrate present in the body consists of the 'sugar' in the blood and tissue fluids, and the glycogen stored in the tissues, principally in the liver and muscles. These substances exist in varying amounts dependent on a large number of factors, such as the amount of carbohydrate ingested, the needs of the organism for carbohydrate, the action of the nervous system, and, in particular, the modifying effects of the adrenal gland elaborated by the endocrine glands. That the cortex of the adrenal gland exerts such effects is now well known. In a recent paper, Harris¹ has said of this relationship:

"Carbohydrate metabolism is most affected by adrenal insufficiency. The absorption is reduced. This reaction coupled with a possible increased utilization, may account for the flattened dextrose tolerance curve found in patients with Addison's disease. The ability to form carbohydrate from non-carbohydrate sources (such as protein) is diminished or abolished. On the other hand, large doses of adrenal extract . . . can raise the liver glycogen considerably above the normal level. The decrease in fasting blood sugar may be accounted for by increased utilization and diminished glycogenesis."

It is to be noted that much of these conclusions is based on results obtained from operations made on animals which has of course adrenal insufficiency within a comparatively short time after operation.

The adult albino rat, however, may live for a comparatively long time after double adrenalectomy, exhibiting meanwhile a chronic type of insufficiency. Even here, not all strains react the same, some being 100% susceptible within a week or two.

Wyman and Walker⁴, using adrenalectomized rats having autoplasmic cortical transplants, showed them to be not unlike normal rats with respect to blood sugar.

Britton and Silvette⁵ demonstrated that an effect of an extract of the adrenal cortex was an increased deposition of liver glycogen in both normal and adrenalectomized rats.

In line with these studies, it seemed pertinent to investigate the levels of blood sugar, liver glycogen, and muscle glycogen, in a series of rats exhibiting various degrees of insufficiency.

4. Wyman, L.C. and B.S.Walker. Am. J. Physiol. 89: 215. 1929

5. Britton, S.W. and H. Silvette. Cold Spring Harbor Symposia Quant. Biol. 5: 357. 1937

In an early survey of the literature, I was impressed with the wealth of accounts of experimental work which exist in the field of carbohydrate metabolism. Much of this work has been reviewed in a number of authoritative and detailed summaries which were useful in comprehending the subject as a whole. A list of some of the more helpful of these reviews is included in footnote 6.

-
6. Shaffer, P.A. *Physiol. Rev.* 3: 394. 1923
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 Newton, W.H. in *Evan's Recent Advances in Physiology*. 6th Ed.
 P. Blakiston's Son + Co. 1939
 Pfiffner, J.J. *Advances in Enzymology*. 2: 325. 1942
 Interscience Publ.

IV. CARBOHYDRATE METABOLISM (GENERAL)

A) DIGESTION

The carbohydrates of the diet consist essentially of starches and cellulose, several disaccharides (sucrose, lactose, and maltose), and a number of monosaccharides, the chief of which is glucose. Previous to absorption, the carbohydrate must either be in the form of the monosaccharide or be broken down into that form. To facilitate the latter process, certain enzymes are provided which under suitable conditions accelerate the process of digestion (or hydrolysis of the larger molecules) and make carbohydrates available to the organism. The exception is cellulose. In mammals, cellulases are lacking, other than those of bacteria. However, amylases and disaccharases are present in the alimentary tract and use is made of them.

The hydrolysis of starch is started in the mouth by salivary amylase, or ptyalin. If the correct pH is maintained, this process may be continued in the stomach. But eventually the salivary amylase is inactivated by the gastric juice. Hydrolysis of the sucrose may be assisted by the hydrochloric acid of the stomach, but, as far as is known, there are no specific carbohydrases present in the stomach juice. The pancreatic juice, however, contains an enzyme which can act on starch and it may be that this pancreatic amylase carries on the major part of the starch-splitting process, since under ordinary conditions, food does not remain for long in the mouth. In addition, the pancreatic juice produces a maltase, which furthers the splitting from maltose to the monosaccharides. The intestinal juice produces three enzymes of parti-

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cular interest, namely invertase, maltase, and lactase. These act respectively on sucrose, maltose, and lactose, producing the constituent monosaccharides. The non-hydrolyzable polysaccharides are excreted in the feces. Thus ultimately one or more of the three monosaccharides glucose, fructose, and galactose may be available for absorption.

B) ABSORPTION

The rate of absorption from the alimentary canal is, within wide limits, independent of the amount of sugar present in the intestinal tract (Cori)⁷. There has also been shown to be a differential rate of absorption for the hexoses, galactose being absorbed the fastest, with glucose being the next. Fructose and mannose are absorbed somewhat more slowly.

Laszt⁸, and Laszt and Stüllmann⁹, have demonstrated that phosphorylation of glucose, fructose, and galactose takes place in the intestinal mucosa, and that those sugars which are not rapidly absorbed, such as arabinose and xylose, involve no phosphorylation in their passage across the intestinal wall. Verzar¹⁰ (1936) claimed that the secretion of the adrenal cortex is an essential agent in the phosphorylation process, but recent attempts to confirm his findings and conclusions have been almost uniformly unsuccessful (Bruce and Wein¹¹, Ochoa and Rossiter¹²).

-
7. Cori, C.F. *Physiol. Rev.* 11: 143. 1931
 8. Laszt, L. *Biochem. Z.* 276: 40. 1935
 9. Laszt, L. and H. Stüllmann. *Biochem. Z.* 278: 401. 1935
 10. Verzar, F. and E.J. McDougall. *Absorption from the Intestine.*
Longmans, Green + Co., New York. 1936
 11. Bruce, H.M. and R. Wein. *J. Physiol.* 98: 375. 1940
 12. Ochoa, S. and R.J. Rossiter. *J. Physiol.* 97: 1P. 1940

C) INTERMEDIARY METABOLISM

1) The blood sugar

Following a carbohydrate meal, there is an increase in the blood sugar. This increase depends in part on the amount of sugar available, its rate of absorption, and the rate at which it is disposed of. Normally, the alterations are never very great, since the regulating mechanisms are constantly operating to maintain a relatively steady level of blood sugar. To determine how much of an increase takes place, it is customary to determine first the fasting level. Cori and Cori¹³ give 99 mgms. per 100 cc. whole blood as the average value for fasting normal rats. Wyman and Walker¹⁴, using a different method for the determination of glucose, found even lower values. They considered the usual range of blood sugar for normal rats in the fasting state to lie between 70 and 100 mgms. per 100 cc. whole blood. Even these values are probably high for the 'true' blood sugar value. Improvements in the techniques of blood protein precipitation have produced filtrates which are almost free from reducing substances other than glucose (Somogyi)¹⁵.

Following glucose ingestion, the blood sugar level may rise to 150 mgms. per 100 cc., but rarely more than that. After a period of several hours, the blood sugar returns to normal values. One or more of several processes may be involved in the disappearance of the glucose.

Among these are diffusion into the tissues, conversion of glucose to

13. Cori, C.F. and G.T. Cori. J. Biol. Chem. 74: 473. 1927

14. Wyman, L.C. and B.S. Walker. Am. J. Physiol. 89: 215. 1929

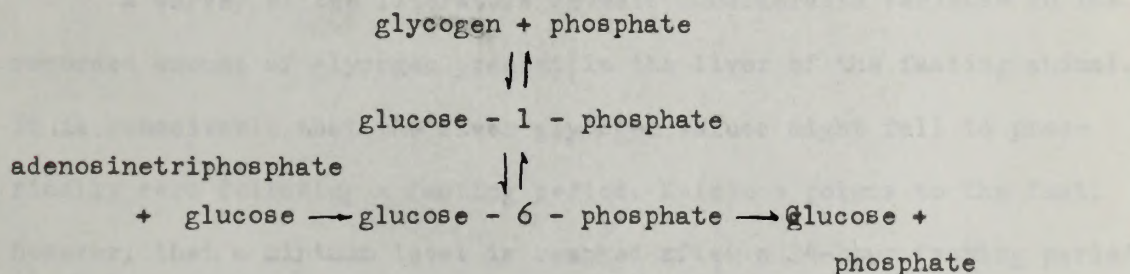
15. Somogyi, M. J. Biol. Chem. 86: 655. 1930

glycogen in either the liver or muscles or both, oxidation of the glucose, its removal by the kidneys if in excess of the renal threshold, or its possible conversion to some other substance, such as fat. In any case, factors of major importance to the carbohydrate metabolism are the liver and muscle glycogen. How this material (glycogen) is formed from glucose, and how the reverse reaction occurs, is to be considered next.

Glucose is either the liver or muscle or both, oxidation of the
glucose, its removal by the kidneys if in excess of the renal threshold,
or its possible conversion to some other substance, such as fat. In any
case, factors of major importance to the carbohydrate metabolism are
the liver and muscle glycogen. Now this material (glycogen) is formed
from glucose, and has the reverse reaction occurs, to be converted
back.

2) The glycogen $\rightleftharpoons$ glucose reaction

The reaction glycogen $\rightleftharpoons$ glucose has been extensively investigated in recent years, and great advances made in our knowledge of the most fundamental aspects of carbohydrate metabolism. Many of the individual steps which have been identified "invitro" are not observed in the intact cell, since the intermediate products do not accumulate in the "in vivo" process. Many reactions are involved in the anaerobic breakdown of carbohydrates which Meyerhof¹⁶ stated . . . "are brought about by phosphorylation of the metabolites". Cori¹⁷ summarized the intermediate steps between glycogen and glucose in the following scheme.



The reaction glycogen $\rightleftharpoons$ glucose can be demonstrated to take place in a test tube in the absence of any of the hormones affecting the rate of this reversible change under physiological conditions (e.g. insulin, adrenalin, or corticosterone). Because of this, Cori (in the same paper) suggested . . . "that these hormones are not essential components of enzyme systems and that their effect of accelerating or inhibiting certain enzymatic reactions is dependent on a more or less intact cell structure".

16. Meyerhof, O. Biol. Symposia. 5: 141. 1941

17. Cori, C.F. Biol. Symposia. 5: 131. 1941

3) Liver glycogen

Soskin¹⁸ regarded the liver function as the prime factor for the maintenance of the normal blood sugar level. The glycogen of the liver represents a reserve of material. As glucose is removed from the blood by the tissues, the liver acts to keep the blood sugar level constant by pouring out glucose from the reserve material. This process of conversion of glycogen to glucose is accomplished with extreme rapidity, and is accelerated by a number of factors, e.g. stimulation by the nervous system, or hormones. Thus while it is probable that a certain amount of hydrolysis may be going on all the time, the rate may differ from time to time.

A survey of the literature reveals considerable variance in the recorded amount of glycogen present in the liver of the fasting animal. It is conceivable that the liver glycogen values might fall to practically zero following a fasting period. Evidence points to the fact, however, that a minimum level is reached after a 24-hour fasting period, whereupon the liver utilizes new material (such as protein) for conversion to glycogen. Thus Barbour¹⁹ et al found a mean of 160 mgms. (per 100 gms. wet liver) of liver glycogen in rats after 24 hours of fasting, and 320 mgms. (per 100 gms.) for animals fasting for 48 hours. Cori and Cori²⁰ obtained similar results, their values for the 24-hour period being 100 mgms. (per 100 gms. wet liver) and for the 48-hour fasting animal, 397 mgms. Other values are given in Table I.

18. Soskin, S. *Physiol. Rev.* 21: 140. 1941

19. Barbour, A.D., L.I. Chaikoff, J.J.R. MacLeod, and M.D. Orr. *Am. J. Physiol.* 80: 243. 1927

20. Cori, C.F. and G.T. Cori. *J. Biol. Chem.* 76: 755. 1928

Table I

LIVER GLYCOGEN OF RATS

(Values in Mgms. per 100 Gms. Wet Liver)

	24 hour fast	48 hour fast
Evans ²¹	207	
Evans ²²	192	
Barbour et al ²³	160	320
Cori and Cori ²⁴	100	397
Nutter ²⁵	34	
Russell ²⁶	30	
Russell and Bennett ²⁷	25	
Long, Katzin, and Fry ²⁸	25	233
Reindollar ²⁹		140
McBride ³⁰		310
Fenn ³¹		200

21. Evans, G. Endocrinology. 29: 731. 1941
22. Evans, G. Am. J. Physiol. 134: 798. 1941
23. Barbour, A.D., L.I. Chaikoff, J.J.R. MacLeod, and M.D. Orr.
Am. J. Physiol. 80: 243. 1927
24. Cori, C.F. and G.T. Cori. J. Biol. Chem. 76: 755. 1928
25. Nutter, P.E. J. Nutrition. 21: 477. 1941
26. Russell, J.A. Am. J. Physiol. 128: 552. 1940
27. Russell, J.A. and L.L. Bennett. Am. J. Physiol. 118: 196. 1937
28. Long, C.N.H., B. Katzin, and E.G. Fry. Endocrinology. 26: 309. 1940
29. Reindollar, W.F. Proc. Soc. Exptl. Biol. and Med. 33: 182. 1935
30. McBride, J.J. J. Biol. Chem. 147: 333. 1943
31. Fenn, W.O. J. Biol. Chem. 128: 297. 1939

It is to be noted that the largest value given is almost ten times greater than the smallest. Doubtless the procedures used were not uniform throughout, but more particularly it lends evidence to the fact that the liver glycogen content can vary considerably under different conditions. Physiological factors affecting these conditions will be discussed under their appropriate headings. It may be noted here that differences in strain, the type of diet, the condition of the animal room, and the general health of the animal will all affect the liver glycogen in fasting conditions. Deuel et al³² reported a diurnal variation in the glycogen content of the liver and suggested that killing of the animals should be performed at the same time of day on any day. Concerning the terminal procedures employed prior to the determination of liver glycogen, there can be no doubt that they exert a marked effect upon the glycogen content found in the liver upon analysis. Cori and Cori³³ stated that . . . "it has been shown repeatedly that killing an animal always causes a considerable loss of muscle glycogen and thereby often leadsto erroneous results". Murphy and Young³⁴, working with several types of anesthesia, demonstrated amytal to have the least effect on carbohydrate metabolism. Cori³⁵ also found this to be true. It may be added that with the death of the animal autolysis of the glycogen begins immediately, so that rapid manipulation of the liver is quite necessary.

In summary, it may be said that the liver acts as a reservoir of

32. Deuel, H.J., Jr., Butts, J.S., J.S.Hallman, S.Murray, and H. Blunden.
J. Biol. Chem. 123: 257. 1938

33. Cori, C.F. and G.T.Cori. Ann. Rev. Biochem. 3: 151. 1934

34. Murphy, C.E. and F.G.Young. J. Physiol. 76: 413. 1932

35. Cori, G.T. Am. J. Physiol. 95: 285. 1930

It is to be noted that the largest value given is almost two times greater than the smallest. Doubtless the procedures used were not uniform throughout, but more particularly it lends evidence to the fact that the liver glycogen content can vary considerably under different conditions. Physiological factors affecting these conditions will be discussed under the appropriate headings. It may be noted here that differences in strain, the type of diet, the condition of the animal, room, and the general health of the animal will all affect the liver glycogen in fasting conditions. Fowl et al.²² reported a diurnal variation in the glycogen content of the liver and suggested that killing of the animals should be performed at the same time of day on any day. Concerning the technical procedures employed prior to the determination of liver glycogen, there can be no doubt that they exert a marked effect upon the glycogen content found in the liver upon analysis. Gort and Gort²³ stated that "... it has been shown repeatedly that killing an animal always causes a considerable loss of muscle glycogen and thereby effecting a marked reduction in results." Murphy and Young²⁴, working with various types of animals, demonstrated again to have the least effect on carbohydrate metabolism. Gort²⁵ also found this to be true. It may be added that with the death of the animal analysis of the glycogen begins immediately, so that rapid manipulation of the liver is quite necessary. In summary, it may be said that the liver acts as a reservoir of glycogen. Fowl et al., Gort, Fowl, Gort, and H. Blumberg. J. Biol. Chem. 124: 197, 1938. 22. Gort, G. and G. Fowl. Ann. Rev. Biochem. 1: 131, 1932. 23. Gort, G. and G. Fowl. J. Physiol. 75: 415, 1932. 24. Murphy, G. and G. Young. J. Physiol. 75: 285, 1930.

sugar, supplying the blood according to its needs. If glucose is provided the blood from some other source (as for example absorption from the intestine) then the liver decreases its output (Soskin)³⁶.

In the healthy animal a certain amount of glycogen is always stored (the source of which is carbohydrate). Glycogen may be metabolized and used. This is the characteristic storage composition of glycogen in the muscle. The chief source of muscle glycogen is the blood sugar. While the liver can transform l-lactic acid into glycogen, it does so less readily than the liver. It takes a longer time. Furthermore, glycogen is removed from the muscle by conversion to lactic acid, thus differing from the liver, where glycogen is removed as glucose. Hence the glycogen apparently comes from the glucose which is converted into glycogen in the liver.

While the value 0.05-0.1% for muscle glycogen profile is wide, usually the muscle glycogen content is very small, even during fasting conditions. In table II are presented values for glycogen of rat muscle, obtained after a 7-hour fasting period. These, while not subject to the marked variations of liver glycogen, are not much affected by such factors as the diet and general health of the animal. Particularly when the animal is under the influence of parathyroid or other factors, where metabolic changes are taking place.

.....

36. Soskin, S. Physiol. Rev. 21: 140. 1941

H. Goss, M.D. J. Nutrition 21: 205, 1941

anyway, supplying the blood according to its needs. If glucose is provided the blood from some other source (as for example absorption from the intestine) then the liver decreases its output (Glicks).

4) Muscle glycogen

Skeletal muscle contains from 0.05 to 1.0 gms. of glycogen per 100 gms. wet muscle, and this is formed from the blood glucose. Since in the healthy animal a certain amount of energy is always needed (the source of which is carbohydrate), glucose must be constantly used up. This in turn necessitates steady deposition of glycogen in the muscle. The chief source of muscle glycogen is the blood sugar. While muscles can transform d-lactic acid into glycogen, it does so less readily than the liver. It seems to prefer glucose. Furthermore, glycogen is removed from the muscle by conversion to lactic acid, thus differing from the liver, where glycogen is removed as glucose. Hence the blood sugar apparently comes from two sources only: 1. from absorbed carbohydrate; 2. from liver glycogen.

While the values 0.05 - 1.0 % for muscle glycogen provide a wide margin, actually the muscle glycogen changes very slowly, even during fasting conditions. In table II are presented values for glycogen of rat muscle, obtained after a 24-hour fasting period. These, while not subject to the marked variability of liver glycogen, are no doubt affected by such factors as the diet and general health of the animal. Particularly would this be true in cases of parasitic infestations of the animals, where constant muscular contractions would take place.

Guest³⁷ states that

"Little variation in muscle glycogen concentration occurs in a blood sugar range between 50 and 100 mgms. %, but above this level the general tendency is toward glycogen storage."

37. Guest, M.M. J. Nutrition. 22: 205. 1941

4) Muscle Glycogen

Skeletal muscle contains from 0.05 to 1.5 gms. of glycogen per 100 gms. wet muscle, and this is formed from the blood glucose. Since in the healthy animal a certain amount of energy is always needed (the source of which is carbohydrate), glucose must be constantly used up. This in turn necessitates a steady deposition of glycogen in the muscle. The chief source of muscle glycogen is the blood sugar. While muscle can transform lactic acid into glycogen, it does so less readily than the liver. It seems to prefer glucose. Furthermore, glycogen is removed from the muscle by conversion to lactic acid, thus differing from the liver, where glycogen is removed as glucose. Hence the blood sugar apparently comes from two sources only: 1. from absorbed carbohydrate; 2. from liver glycogen.

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TABLE II

"Little variation in muscle glycogen concentration occurs in a blood sugar range between 75 and 100 mgms. % but above this level the general tendency is toward glycogen storage."

Table II

MUSCLE GLYCOGEN OF RATS

(After 24 hours of fasting)

(Values in Mgms. per 100 Gms. Wet Muscle)

Buell, Anderson, and Strauss ³⁸	456
Russell and Bennett ³⁹	510
Long, Katzin, and Fry ⁴⁰	520
Evans ⁴¹	521
Russell ⁴²	524
Nutter ⁴³	571

38. Buell, M.V., I.A. Anderson, and M.B. Strauss. Am. J. Physiol. 116: 274. 1936

39. Russell, J.A. and L.L. Bennett. Am. J. Physiol. 118: 196. 1937

40. Long, C.N.H., B. Katzin, and E.G. Fry. Endocrinology. 26: 309. 1940

41. Evans, G. Am. J. Physiol. 134: 798. 1941

42. Russell, J.A. Am. J. Physiol. 128: 552. 1940

43. Nutter, P.E. J. Nutrition. 21: 477. 1941

Table II

MEAN BLOOD SUGAR

(After 24 hours of fasting)

(Values in mgm. per 100 ccm. of blood)

456	Shell, Anderson, and Strasser ²⁸
310	Russell and Bennett ³²
320	Long, Katzin, and V.Y. ⁴⁰
321	Evans ⁴¹
324	Russell ⁴²
371	Mutter ⁴³

28. Shell, H.V., L.A. Anderson, and E. Strasser. Am. J. Physiol. 116: 374, 1936

32. Russell, J.A. and W. Bennett. Am. J. Physiol. 113: 130, 1937
 40. Long, C.M., B. Katzin, and E.C. V.Y. Endocrinology. 35: 309, 1940
 41. Evans, G. Am. J. Physiol. 134: 193, 1941
 42. Russell, J.A. Am. J. Physiol. 123: 737, 1940
 43. Mutter, P.A. J. Nutrition. 21: 317, 1941

5) Hyperglycemia

The term 'hyperglycemia' has been used to denote an increased level of blood sugar above the fasting level. Such a condition may be either temporary, as following the ingestion of abnormally large quantities of glucose, or it may be lasting if the mechanisms for utilization of the glucose are inadequate.

a) Influence of the nervous system

The liver is richly supplied with nerves originating from the sympathetic division of the autonomic system. Stimulation of these produces a rapid breakdown of the liver glycogen, and a transfer of glucose to the blood stream takes place, thereby raising the blood sugar level.

b) Effect of the adrenal medulla

The medullary portion of the adrenal gland elaborates a substance known as adrenaline. When this substance is poured forth from the gland, or is injected into the blood stream by other methods, a rapid mobilization of blood sugar takes place.

Thus the sympathetic nervous system and the adrenal medulla form a part of the 'emergency' mechanism of the animal and must be carefully controlled in any experimentation on carbohydrate metabolism. The effect of anesthesia has already been mentioned. All of these effects cause a depletion of liver glycogen, and usually a concomitant rise in blood sugar. Careless handling of the animal prior to the terminal procedures involve these effects, and frequently lead to erroneous results.

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D) REGULATION OF CARBOHYDRATE METABOLISM

BY GLANDS OF INTERNAL SECRETION1) Insulin

The problems of the diabetic state have been subject to intensive investigation ever since the discovery by von Mering and Minkowski⁴⁴ that removal of the pancreas resulted in a rapid rise in blood sugar with a 'spilling over' into the urine. They observed, in dogs from which they had removed the pancreas, a persistent glycosuria, symptoms of marked hunger and thirst, emaciation in spite of liberal feeding, and finally death in the course of a few weeks. They reported that to obtain these results, extirpation of the pancreatic gland must be complete; a small portion remaining was sufficient to keep the animal in good health. Many studies have made use of this observation, the experimental animal being partially depancreatized, instead of having the gland completely removed.

These symptoms closely simulate those present in the human patient suffering from disease of the pancreas. In both experimental diabetes, and the disease in man, a 'wasting away' takes place, and there is considerable loss in liver and muscle glycogen. The slight difference in the glucose content of the arterial and venous blood of an organ indicates that utilization of glucose by the tissues is much reduced from the normal. The most obvious features of the diabetic state are hyperglycemia and glycosuria.

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 44. von Mering, J. and O. Minkowski. Arch. f. exper. Path. u. Pharmacol. 26: 371. 1889

2) REGULATION OF CARBOHYDRATE METABOLISM

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1) Insulin

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.....
 40. von Mering, J. and O. Minowski. Arch. f. exper. Path. u. Pharm.
 1891. 26: 571. 1899

In 1922, Banting and Best⁴⁵ isolated from the pancreas the hormone known as insulin. Injection of this substance produces a rapid drop in the blood sugar. Why it (the blood sugar) accumulates in the absence of the pancreas, and falls so rapidly following injection of insulin is a much debated subject. Administration of insulin to the 'diabetic' restores the carbohydrate metabolism to normal. Combustion of carbohydrate is accelerated, and the liver and muscle glycogen stores return to normal.

Waters and Best⁴⁶ have reviewed the action of insulin, and state that it decreases gluconeogenesis from protein, encourages the formation of glycogen, and increases the combustion of carbohydrate. Drury⁴⁷ regards the important action of insulin as brining about the storage of sugar at the time it is absorbed, and suggests that it accelerates the transformation of glucose to fat. These findings need further confirmation.

Soskin and Levine⁴⁸ believe the primary action of insulin to be the regulation of blood sugar, by affecting the rate of sugar output by the liver and the rate of sugar uptake by the peripheral tissues. They suggested that insulin is not primarily concerned with the oxidative breakdown of carbohydrates.

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45. Banting, F.G. and C.H. Best. J. Lab. Clin. Med. 7: 251. 1922

46. Waters, E.T. and C.H. Best. J. Am. Med. Assoc. 117: 852. 1941

47. Drury, D.R. Am. J. Physiol. 131: 536. 1940

48. Soskin, S. and R. Levine. Am. J. Physiol. 129: 782. 1940

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43. Hastings, P. B. and J. B. Best. J. Lab. Clin. Med. 7: 521, 1922
44. Hastings, P. B. and J. B. Best. J. Lab. Clin. Med. 17: 521, 1931
45. Givry, D. R. Am. J. Physiol. 151: 555, 1940
46. Boodin, S. and A. Levine. Am. J. Physiol. 127: 732, 1940

Working with liver slices, Corey and Britton⁴⁹ found a loss of liver glycogen after treatment with insulin. They considered insulin to be concerned chiefly with the formation of muscle glycogen.

Hechter et al⁵⁰ regarded insulin as influencing only the rate at which glucose enters the cell from without, while the work of Gemmill⁵¹ suggests that the hormone is actively concerned with glycogen formation from glucose.

The weight of the evidence suggests that insulin is chiefly concerned, either directly or indirectly, with glycogen storage in the peripheral tissues. Additional investigations on the specific action of insulin need to be carried out.

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49. Corey, E.L. and S.W. Britton. *Am. J. Physiol.* 131: 783. 1941
50. Hechter, O., R. Levine, and S. Soskin. *Proc. Soc. Exper. Biol. Med.* 46: 390. 1941
51. Gemmill, C.L. *Am. J. Physiol.* 133: P291. 1941

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12. Gorry, E. L. and H. B. Briston. *Am. J. Physiol.* 134: 752, 1941.
 13. Heston, G. L., R. Levine, and S. Neelam. *Proc. Soc. Exper. Biol. Med.* 48: 200, 1942.
 14. Gemmill, G. L. and J. F. Fyfe. *Proc. Soc. Exper. Biol. Med.* 48: 200, 1942.

2) Influence of the anterior pituitary

Houssay⁵², and Young⁵³ have written excellent summaries of the part the pituitary is believed to play in carbohydrate metabolism. Chief among its properties in this respect is its antagonistic action to insulin. The anterior lobe of the pituitary markedly affects carbohydrate metabolism, stimulating those processes which, if driven to excess, lead to diabetes. This is known as the 'diabetogenic' action of the anterior lobe. Apparently, the anterior lobe extract favors gluconeogenesis (i.e. formation of carbohydrate from non-carbohydrate substances), and counteracts the peripheral action of insulin on the tissues. It is interesting to note that in the 'Houssay' animal (one from which both the pancreas and the pituitary have been removed) the carbohydrate metabolism somewhat resembles that of the normal state. Newton⁵⁴ suggests that the real action of the anterior pituitary in the normal animal is to bring about a greater utilization of fat and a sparing of protein. Thus there seems to be a delicate balance between the action of the anterior lobe of the pituitary and the pancreatic hormone. It may be that the pituitary exerts its effect in two ways: first, by its stimulating effect on the adrenal cortex (to be discussed); and secondly, by a direct action in stimulating gluconeogenesis in the liver.

.....

52. Houssay, B.A. Endocrinology. 30: 884. 1942

53. Young, F.G. Endocrinology. 26: 345. 1940

54. Newton, W.H. in Evan's Recent Advances in Physiology. 6th Ed.
P. Blakiston's Son + Co. 1939

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22. Hewson, R.A. *Endocrinology*. 30: 634. 1942.
23. Long, J.C. *Endocrinology*. 30: 393. 1940.
24. Hewson, R.A. in *Recent Advances in Physiology*. 6th Ed. Chapman & Co. 1939.

3) The adrenal gland

a) The medulla

The effects of adrenaline have already been discussed. The primary site of its action is the liver, where glycogenolysis is greatly accelerated following an increase in adrenaline output.

b) The cortex

The investigation herein to be described is concerned with the relationship of the adrenal cortex to carbohydrate metabolism in rats with transplanted adrenals. I shall therefore consider this portion of the gland in more detail in the following section.

3) The adrenal gland

a) The medulla

The effects of adrenaline have already been discussed. The primary site of its action is the liver, where glycogenolysis is greatly accelerated following an increase in adrenaline output.

b) The cortex

The investigation has to be described in connection with the relationship of the adrenal cortex to carbohydrate metabolism in rats with transplanted adrenals. I shall therefore consider this portion of the report in more detail in the following section.

V. RELATION OF THE ADRENAL CORTEX TO CARBOHYDRATE METABOLISM

In 1855, an English physician named Thomas Addison⁵⁵ described a condition in the human which was associated with degenerative changes of the adrenals. The disease has since been termed 'Addison's disease'. His description of it was so clearly presented, and has been modified so slightly in view of later investigations, that it is worth while to quote him on the subject. Thus:

"The leading and characteristic features of the morbid state to which I would direct attention are anemia, general languor and debility, remarkable feebleness of the heart's action, irritability of the stomach, and a peculiar change of colour in the skin, occurring in connection with a diseased condition of the 'supra-renal capsules'."

Best and Taylor (1939)⁵⁶ state that the chief features of disease of the adrenal cortex in man are:

". . . (a) muscular weakness and languor, (b) low blood pressure and reduced circulation rate, (c) gastro-intestinal disturbances, loss of appetite (anorexia), hypochlorhydria and vomiting, (d) pigmentation of skin and mucous membranes, bronzing, tanning or a dirty brown discoloration of the former being a classical symptom of the disease, (e) lowered metabolic rate, subnormal temperature, sodium loss and a rise in serum potassium, reduced blood volume (plasma loss), dehydration and loss of weight, (f) renal insufficiency with consequent rise in blood non-protein nitrogen, (g) depression of the sexual functions, (h) hypoglycemia may occur and be the immediate cause of death."

Addison's disease unless treated by replacement therapy is almost invariably fatal within from one to three years.

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55. Addison, T. From 'A collection of the published writings of the late Thomas Addison, M.D.'. The New Sydenham Society. London. 1868

56. Best, C.H. and N.B. Taylor. The Physiological Basis of Medical Practice. 2nd Ed. The Williams + Wilkins Co. 1939

Experimental extirpation of both adrenal glands in animals produces a condition similar to Addison's disease in the human. If the experimental animal is in good health at the time of operation, and the operative techniques are without fault, the healthy condition will prevail for several days, or sometimes more than a week, after adrenalectomy. Good care of the animal, particularly with regard to warm living quarters and tempting food, are necessary to obtain the best survival periods. From one to three days before death supervenes, the animal refuses to eat, either in part or in whole. Muscular weakness may develop; vomiting and diarrhea may occur. Muscular twitchings and convulsions are not uncommonly seen. The heart beat becomes slow and irregular, and the body temperature falls a few degrees below normal. Gradually an apathetic condition is exhibited by the animal; little interest is taken in the surroundings. This condition lasts a few hours and finally death ensues. If an autopsy is performed immediately following, the blood appears thick, and the tissues dehydrated. Rats differ in their survival period according to strain. Many live for long periods after adrenalectomy. However, a progressive emaciation sets in, and often these animals die from intercurrent infections.

In 1930, Swingle and Pfiffner⁵⁷ reported a method of preparing adrenal extracts sufficient to maintain indefinitely the lives of adrenalectomized animals. At about the same time, Hartman and Brownell⁵⁸ reported an equally successful method. Other methods soon followed.

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57. Swingle, W.W. and J.J. Pfiffner. Science. 71: 321. 1930

58. Hartman, F.A. and K.A. Brownell. Proc. Soc. Exper. Biol. + Med. 27: 938. 1930

Thus it became possible, by subjecting animals to adrenalectomy and then partially supplying their hormonal needs by means of an extract, to create a condition of 'experimental chronic adrenal deficiency'.

Several schools of thought soon advanced theories concerning the chief function of the adrenal cortex. Each school considered the cortical function to be concerned with a particular primary factor, the other factors appearing secondarily as a result of the first. Thus by 1933⁵⁹, Britton and Silvette had suggested that the adrenal cortex prepotently regulates carbohydrate metabolism. Hartman and his school considered that the cortex produces a general tissue hormone, while Swingle et al believe it to control the circulating blood volume. Wyman and tum Suden⁶⁰, in a comprehensive study on "The blood volume of the rat in suprarenal insufficiency, anaphylactic shock and histamine shock" suggested that the ". . . cortex is concerned in the steady maintenance of certain bodily conditions . . .", and offered evidence supporting the theory that ". . . some influence of the cortex prevents the accumulation of toxic products of metabolism in the body by maintaining normal channels of elimination or destruction . . .".

Considerable debate ensued as to which was the correct theory, and each group offered supporting evidence for its side of the argument. However, real progress was not made until investigation into the chemical nature of the adrenal cortical extract showed it to be not one substance, but a mixture of active (and inactive) substances which produced the varying effects.

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59. Britton, S.W. and H. Silvette. Science. 77: 367. 1933

60. Wyman, L.C. and C. tum Suden. Am. J. Physiol. 94: 579. 1930

A) ADRENOCORTICAL HORMONES

1) Nomenclature and structure

As a result of many recent investigations in adrenal physiology, it has become increasingly clear that certain of the compounds isolated from the adrenal cortex differ significantly in their effects on the animal organism. At least five, and possibly a sixth hormone having cortin activity, have been isolated from adrenal extracts. Mason⁶¹ states: "The chemical work on the adrenal gland has led to the isolation of more than twenty crystalline substances which are closely related."

Many difficulties exist in correlating and understanding the physiological activities of these fractions, due in part to the relatively small quantities of the pure hormones (other than desoxycortico-sterone) which are available for study. Many methods of assay are used, and not infrequently vary in principle, making comparisons between the methods difficult. Loeb's⁶² discovery of the close association of sodium metabolism with adrenal function pointed to the fact that careful control of diet is a prerequisite in assay procedures.

In the following section, I propose to review briefly certain of the compounds found in adrenal cortical extracts, and to discuss their possible relation to carbohydrate metabolism.

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61. Mason, H.L. Proc. Staff Meet. Mayo Clin. 15: 289. 1940

62. Loeb, R.F. Proc. Soc. Exper. Biol. + Med. 30: 808. 1933

2) AGGREGATION OF RED BLOOD CELLS

3) AGGREGATION OF RED BLOOD CELLS

As a result of many recent investigations in adrenal physiology, it has become increasingly clear that certain of the compounds released from the adrenal cortex affect significantly its target effects on the animal organism. In fact, the possibility of a direct hormonal action of certain adrenal steroids has been established from adrenal extracts. Hansen et al. (1950) have shown that the adrenal gland has led to the production of a series of new steroid hormones which are closely related to the known adrenal steroids. These new hormones are characterized by a high degree of biological activity and are capable of exerting effects on the physiological activities of many tissues. One of the most important of these is the effect on the growth of the animal organism. Other than these, there are also compounds which are available for study. Many methods of assay are available and not infrequently vary in principle, making comparisons between the methods difficult. The discovery of the close association of certain adrenal steroids with adrenal function points to the fact that careful study of this is a very valuable in many respects.

In the following section I propose to review briefly certain of the compounds found in adrenal cortical extracts, and to discuss their possible relation to endocrine metabolism.

Dr. Hansen, M.D., Fred. Grant, M.D., Mayo Clinic, 1950
Dr. Hansen, M.D., Fred. Grant, M.D., Mayo Clinic, 1950

In the period 1920 - 30, Rogoff and Stewart⁶³ tested numerous extracts of the adrenal cortex, and demonstrated that the lives of completely adrenalectomized animals could be prolonged by the injection of a cortical extract combined with saline transfusion. Hartman⁶⁴ and his colleagues also obtained a cortical extract capable of definitely prolonging the survival time of adrenalectomized animals, and, in 1928, proposed the name 'cortin' for the hormone of the adrenal cortex necessary for the maintenance of life in the adrenalectomized animal. This term (cortin) came more or less into general usage, and has been employed synonymously with 'adrenal cortical extract'.

In 1936, Kendall⁶⁵ and his associates isolated several fractions from an adrenal gland extract, which were arbitrarily designated fractions A, B, C, et cetera. At about the same time, Reichstein⁶⁶ described isolation of compounds of similar or like nature, but complicated the situation by varying the terminology used, viz., H, C, Fa, M, D, et cetera. As was to be expected, confusion in the use of these terms, arbitrarily chosen for the convenience of the individual laboratory, was the result, and an amplification and correlation of them became necessary. Reviews and summaries of the nomenclature and structure

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63. Stewart, G.N. and J.M. Rogoff. Science. 66: 327. 1927

64. Hartman, F.A., K.A. Brownell, W.E. Hartman, G.A. Dean, and C.G. MacArthur. Am. J. Physiol. 86: 353. 1928

65. Mason, H.L., C.S. Meyers, and E.C. Kendall. J. Biol. Chem. 114: 613. 1936

66. Reichstein, T. Helv. Chim. Acta. 20: 953. 1937

have been made by leading workers in the field, among them being Kendall, Reichstein, Strain, Wintersteiner and Smith, Mason, Ingle, Pfiffner, and Koch. (Footnote 67)

.....

67. Kendall, E.C. J. Biol. Chem. Proc. 133: 51. 1940

Endocrinology. 30: 853. 1942

Reichstein, T. in Ruzicka, L. and W. Stepp., "Ergebnisse der Vitamin- und Hormonforschung". Leipzig. 1: 334. 1938

Strain, W.H. in Gilman, H. "Organic Chemistry". New York. 2: 1220. 1938

Wintersteiner, O. and P.E. Smith. Ann. Rev. Biochem. 7: 253. 1938

Mason, H.L. Proc. Staff Meet. Mayo Clin. 15: 289. 1940

Ingle, D.W. Endocrinology. 31: 419. 1942

Pfiffner, J.J. in "Advances in Enzymology". 2: 325. 1942

Koch, F.C. Ann. Rev. Biochem. 9: 334. 1940

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 Kowalski, Holczer, Strain, Winterstein and Smith. Smith, Strain,
 Winterstein, and Kowalski. (Inorganic 2)

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1. Kowalski, E. C. J. Biol. Chem. 193, 21, 1940
 Biochemistry, 30: 1935, 1936
 Holczer, E. In Holczer, E. and A. Strain, "Organic Chemistry of the
 Nitrogen and Phosphorus Compounds", Vol. 1, 1935
 Strain, W. H. In Strain, E. "Organic Chemistry", New York
 1935, 1936
 Winterstein, J. and E. Smith. Ann. Rev. Biochem. 7: 257, 1938
 Smith, E. C. J. Biol. Chem. 193, 21, 1940
 Smith, E. C. "Biochemistry", 31: 193, 1942
 Winterstein, J. In "Advances in Biochemistry", 2: 25, 1942
 Smith, E. C. and E. Smith. J. Biol. Chem. 193, 21, 1940

It has been shown that the several substances isolated from the adrenal cortex belong to the same class of substances as the sterols and bile acids. A characteristic of all these substances is their basic chemical structure, consisting of a system of four rings, the cyclopentano-phenanthrene ring system. Any substance containing this ring is called a 'steroid' (like sterol). Thus the adrenal compounds are steroids. The ring structure, typical of the steroids, is shown in figure 1.

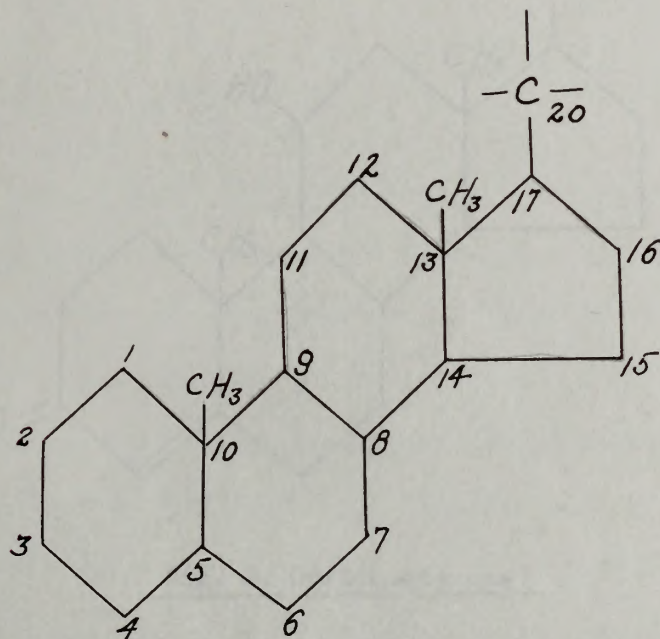


Fig. 1. Cyclopentano-phenanthrene ring system

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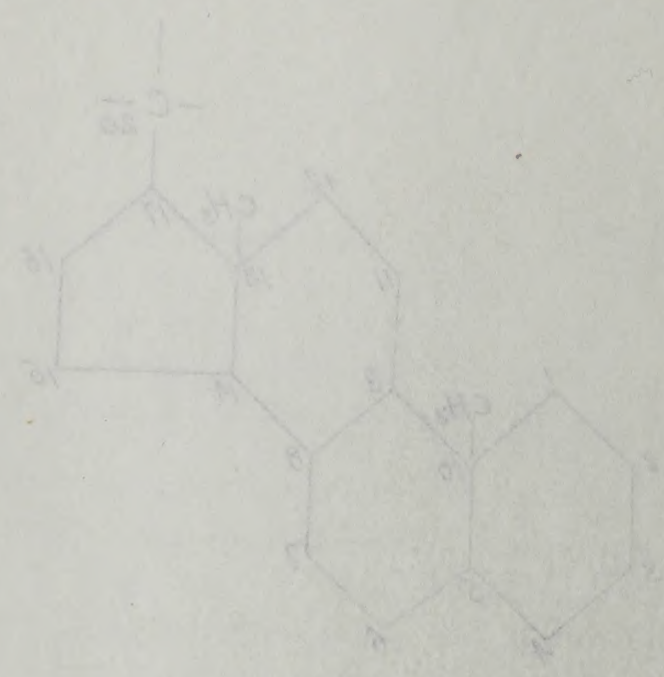


Fig. 1. Cyclopentane-phenanthrene ring system

Reichstein⁶⁸ isolated the first compound from the adrenal gland which had significant cortin activity. This he named 'corticosterone'. Kendall⁶⁹ and co-workers reported a short time later an independent isolation of the same substance. Both groups of workers, using different methods, concurred in the structure of corticosterone (shown in figure 2).

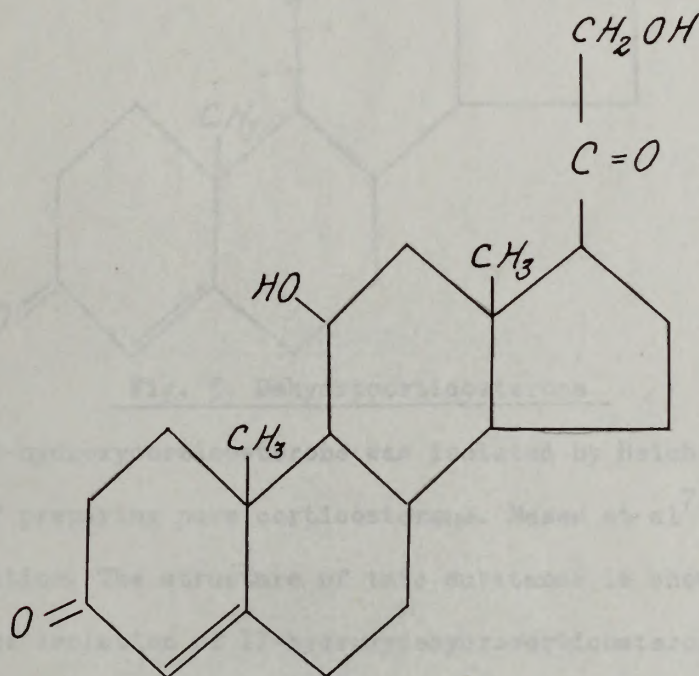


Fig. 2. Corticosterone

As other cortin-active substances were isolated, they were named according to their structural relation to corticosterone.

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68. Reichstein, T. Helv. Chim. Acta. 20: 953. 1937

69. Mason, H.L., W.M.Hoehn, B.F.MacKenzie, and E.C.Kendall.
J. Biol. Chem. 120: 719. 1937

Thus, dehydrocorticosterone, isolated at the same period by the same groups of investigators, has the structural formula shown in figure

3.

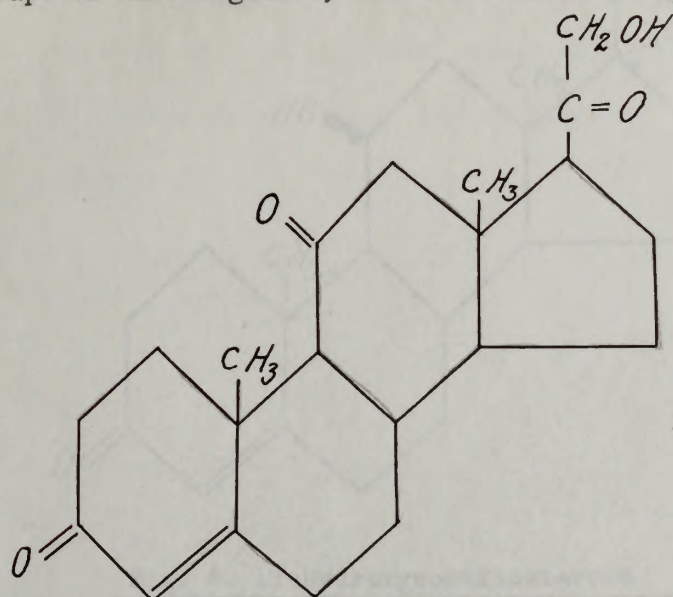


Fig. 3. Dehydrocorticosterone

17-hydroxycorticosterone was isolated by Reichstein⁷⁰ in the course of preparing pure corticosterone. Mason et al⁷¹ also achieved its isolation. The structure of this substance is shown in figure 4.

The isolation of 17-hydroxydehydrocorticosterone was accomplished by Wintersteiner and Pfiffner⁷² in, 1936, and also by Reichstein⁷⁰, but they failed to recognize its physiological activity. At about the same time Mason et al⁷³ isolated, and demonstrated that cortin activity was possessed by this compound (structure shown in figure 5).

70. Reichstein, T. *Helv. Chim. Acta.* 20: 953. 1937

71. Mason, H.L., W.M.Hoehn, and E.C.Kendall.
J. Biol. Chem. 124: 459. 1938

72. Wintersteiner, O. and J.J.Pfiffner. *J. Biol. Chem.* 116: 291. 1936

73. Mason, H.L., C.S.Meyers, and E.C.Kendall.
J. Biol. Chem. 114: 613. 1936

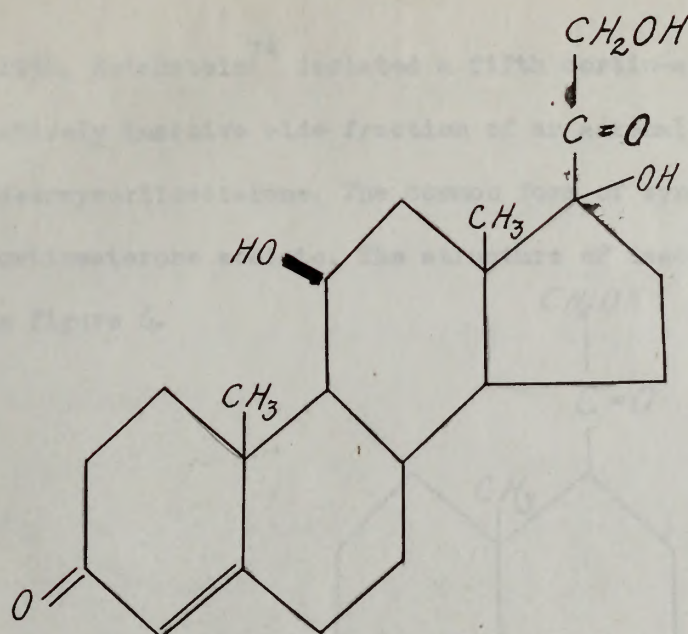


Fig. 4. 17-hydroxycorticosterone

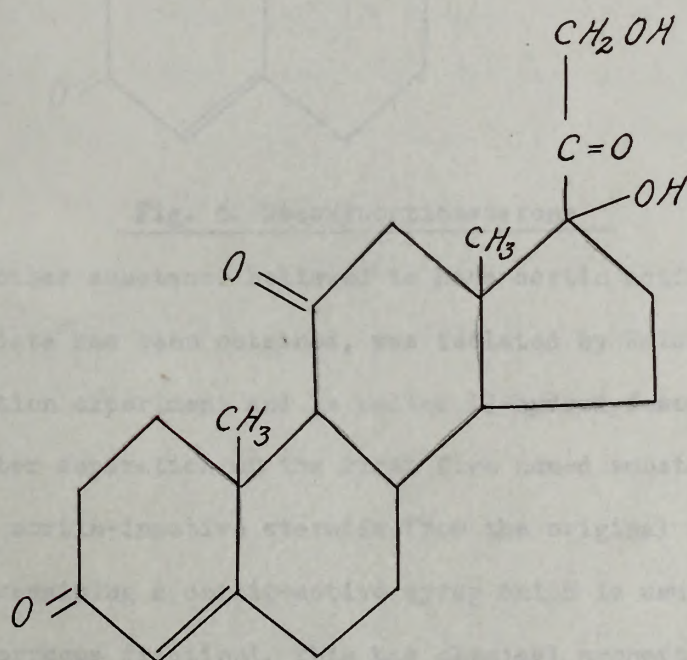


Fig. 5. 17-hydroxydehydrocorticosterone

In 1938, Reichstein⁷⁴ isolated a fifth cortin-active substance from a relatively inactive side fraction of an adrenal extract. This was named desoxycorticosterone. The common form of synthetic compound is desoxycorticosterone acetate. The structure of desoxycorticosterone is shown in figure 6.

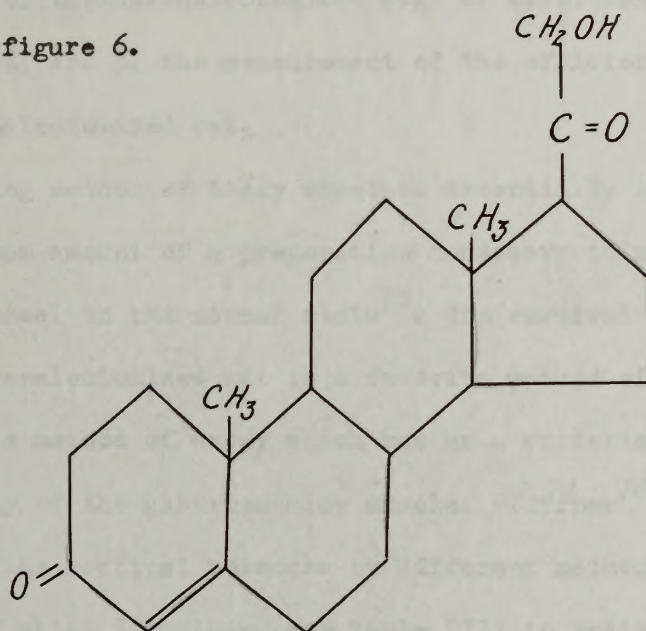


Fig. 6. Desoxycorticosterone

Another substance believed to have cortin activity, but on which no data has been obtained, was isolated by Reichstein in the same fractionation experiment and is called 17-hydroxydesoxycorticosterone.

After separation of the first five named substances together with many cortin-inactive steroids from the original cortical extract, there is remaining a cortin-active syrup which is usually referred to as the 'amorphous fraction'. This has chemical properties closely resembling those of the isolated crystalline hormones, e.g. solubility, specific ultraviolet absorption, et cetera.

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74. Reichstein, T. and J. v.Euw. Helv. Chim. Acta. 21: 1197. 1938

2) Assay and comparative effects

A variety of assay procedures for the evaluation of cortin activity have been suggested. Three in particular have been most used: 1. maintenance of the adrenalectomized dog; 2. maintenance of the adrenalectomized rat; and 3. the measurement of the efficiency of the muscle of the adrenalectomized rat.

The dog method of assay consists essentially in the determination of the minimum amount of a preparation necessary to maintain the adrenalectomized animal in the normal state⁷⁵. The survival and growth of the immature adrenalectomized rat is a favorite method of assay⁷⁶. Ingle⁷⁷ has devised a method of assay which has as a criterion of activity the work capacity of the gastrocnemius muscle. Pfiffner⁷⁸ has tabulated the activity of the cortical hormones by different methods of assay, a modification of which I include (see table III) to indicate the differences in physiological action of these hormones on 'maintenance of life' and 'muscle efficiency'. It will be noted that those having the least effect on 'maintenance of life' appear to exert the greatest activity in respect to 'muscle efficiency'.

.....
75. Pfiffner, J.J., Swingle, W.W. and H.M. Vars.
J. Biol. Chem. 104: 701. 1934

76. Grollman, A. and W.M. Firor. J. Biol. Chem. 100: 429. 1933

77. Ingle, D.W. Am. J. Physiol. 116: 622. 1936

78. Pfiffner, J.J. in 'Advances in Enzymology'. 2: 325. 1942

Table III

COMPARISON OF ACTIVITY OF THE CORTICAL HORMONES BY DIFFERENT
METHODS OF ASSAY (after PFIFFNER)

(Numbers indicate the order of activity. 1 = greatest; 4 = least)

Compound	Maintenance of Life		Muscle Efficiency (Rat)
	Dog	Rat	Work Output
Corticosterone	3	2	1
17-Hydroxycorticosterone	4	3	1
Dehydrocorticosterone	3	2	1
17-Hydroxydehydrocorticosterone	4	3	1
Desoxycorticosterone	2	2	3
Amorphous Fraction	1	1	2

Table III

COMPARISON OF ACTIVITY OF THE CORTICAL HORMONES BY DIFFERENT
METHODS OF ASSAY (after WILLIAMS)

(Numbers indicate the order of activity. 1 = greatest; 4 = least)

Compound	Maintenance of Life		Muscle Efficiency (Rat)
	Hog	Rat	
Corticosterone	2	2	1
17-Hydroxycorticosterone	4	3	1
Dehydrocorticosterone	3	2	1
17-Hydroxydehydrocorticosterone	4	3	1
Dehydroepiandrosterone	2	2	2
Androgenic Fraction	1	1	2

The comparative effects of these crystalline substances on carbohydrate metabolism have been studied extensively since evidence was produced which showed the cortical hormones varied significantly in their effects on this function. Britton and Silvette⁷⁹ early demonstrated that adrenal cortical extract was necessary for deposition of liver glycogen. Long⁸⁰ and his colleagues observed the diabetogenic effect of cortical extract in the partially depancreatized rat, and that glycosuria was markedly stimulated by corticosterone, dehydrocorticosterone and 17-hydroxycorticosterone, but that in comparable dosage desoxycorticosterone had little or no effect. Seyle and Dosne⁸¹ suggested that cortin prevents the hypoglycemic action of insulin (or the decrease in blood sugar by removal of hepatic glycogen stores) by stimulating gluconeogenesis in the liver.

Wells⁸², using the fasting phlorizinized adrenalectomized rat demonstrated that corticosterone and 17-hydroxycorticosterone restore gluconeogenesis to an essentially normal level, while desoxycorticosterone and the amorphous fraction have little or no effect. Thorn⁸³ and his colleagues observed that cortical extract, corticosterone, and 17-hydroxydehydrocorticosterone cause the adrenalectomized rat to respond as a normal animal to phlorizin poisoning. Desoxycorticosterone is without effect. Extension of these findings to observations on

79. Britton, S.W. and H.Silvette. Cold Spring Harbor Symp. Quant. Biol. 5: 357. 1937

80. Long, C.N.H., B. Katzin, and E.G.Fry. Endocrinology. 26: 309. 1940

81. Seyle, H. and C.Dosne. Am. J. Physiol. 128: 729. 1940

82. Wells, B.B. PROC. Staff Meet. Mayo Clin. 15: 294. 1940

83. Thorn, G.W., D.Kuhlman, G.Delbue, G.F.Koeppf, and R.A.Lewis. Endocrinology 27: 971. 1940

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is without effect. Extension of these findings to operations on

79. Britton, S.W. and R. Oliver. Cold Spring Harbor Symp. Quant. Biol. 2: 357, 1937.
80. Long, C.A.H., E. Kasten, and R.B. Fry. Endocrinology. 53: 302, 1940.
81. Bayle, R. and C. Deane. Am. J. Physiol. 128: 739, 1940.
82. Wells, S.W. Proc. Staff Meet. Mayo Clin. 13: 324, 1934.
83. Thorn, C.W., S. Kuhlman, G. Deane, G.F. Koepf, and R.A. Deane. Endocrinology. 27: 971, 1940.

patients suffering with Addison's disease were carried out by this group of investigators⁸⁴. They found in those patients exhibiting symptoms of a disturbed carbohydrate metabolism, e.g. hypoglycemia, that cortical extract, corticosterone, or 17-hydroxydehydrocorticosterone would correct the condition, but that desoxycorticosterone was ineffective. Soffer, Engel, and Oppenheimer⁸⁵, treating Addison's disease with desoxycorticosterone acetate, observed that it readily restored the blood electrolyte pattern, and increased the blood pressure, but exercised no effect on carbohydrate metabolism. Wilder⁸⁶ concludes that

"Desoxycorticosterone acetate, powerful as it is as a regulator of the disturbed salt and water balance of Addison's disease, has been shown to be lacking in that activity of the adrenal cortex which has to do with neoglucogenesis and the maintenance during fasting of a continuous supply of glucose to the tissue".

Gratten and Jensen⁸⁷ studied the various adrenal cortical principles to determine their influence on carbohydrate metabolism. They did this by observing their anti-insulin effect and their influence on liver glycogen. They concluded that corticosterone, 17-hydroxycorticosterone, and 17-hydroxydehydrocorticosterone have a distinct anti-insulin effect, and that their presence leads to the deposition of liver glycogen, but that desoxycorticosterone has practically no effect. These investigators later demonstrated that the anti-insulin effect of

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84. Thorn, G.W., G.F.Koepf, R.A.Lewis, and E.F.Olsen.
J. Clin. Investigation. 19: 813. 1940

85. Soffer, L.J., F.L.Engel, and B.S.Oppenheimer.
J. Am. Med. Assoc. 115: 1860. 1940

86. Wilder, R.M. Proc. Staff. Meet. Mayo Clin. 15: 273. 1940

87. Grattan, J.F. and H.Jensen. J. Biol. Chem. 135: 511. 1940

patients suffering with Addison's disease were carried out by this group of investigators.⁸⁴ They found in these patients exhibiting symptoms of a disturbed carbohydrate metabolism, e.g. hypoglycemia, that cortical extract, corticosterone, or 17-hydroxycorticosterone would correct the condition, but that deoxycorticosterone was ineffective.⁸⁵ Böttcher, Engel, and Goppensheimer, treating Addison's disease with deoxycorticosterone acetate, observed that it readily restored the blood electrolyte pattern, and increased the blood pressure, but exerted no effect on carbohydrate metabolism. Wilber⁸⁶ concludes that

"deoxycorticosterone acetate, powerful as it is as a regulator of the disturbed salt and water balance of Addison's disease, has been shown to be lacking in that activity of the adrenal cortex which has to do with neoglucogenesis and the maintenance during fasting of a continuous supply of glucose to the tissue."

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84. Thorn, C.W., B.F. Joshi, E.A. Loria, and E.F. Olsen. J. Clin. Investigation. 19: 615. 1940.
85. Böttcher, L.J., E.L. Engel, and E.G. Goppensheimer. J. Am. Med. Assoc. 115: 1930. 1940.
86. Wilber, R.A. Proc. Natl. Acad. Sci. 27: 1940.
87. Gratten, L.F. and L. Jensen. J. Biol. Chem. 155: 511. 1940.

the anterior pituitary is produced by the adrenotropic factor and is mediated through the adrenal cortex.⁸⁸ In contrast to these results Harrison and Harrison⁸⁹ reported that administration of desoxycorticosterone acetate prevents the fall of blood sugar in fasted adrenalectomized rats. It may be that desoxycorticosterone influences carbohydrate metabolism indirectly through its effect on electrolyte balance. Seyle and Bassett⁹⁰, studying the effect of desoxycorticosterone on water and chloride metabolism in the intact rat, found a marked diuresis accompanied by an increased chloride excretion.

Kendall⁹¹ expressed the view that desoxycorticosterone is the only adrenocortical principle capable of lowering the concentration of serum potassium, and this factor is mainly involved in the regulation of the electrolyte balance. In the same paper, he states that carbohydrate metabolism is most affected by corticosterone and dehydrocorticosterone, less by 17-hydroxydehydrocorticosterone, that desoxycorticosterone has very little effect, and the amorphous fraction still less. A comparison of the above mentioned effects with the structural arrangement of the several hormones pictured in figures 2,3,4,5, and 6, would indicate that only those hormones having an oxygen atom at C₁₁ exert an effect on carbohydrate metabolism.

In conclusion, the effects of the hormones discussed in this section are compared in summary form in table IV.

88. Grattan, J.F. and H.Jensen. J. Biol. Chem. 135: 511. 1940

89. Harrison, H.E. and H.C.Harrison.

Proc. Soc. Exper. Biol. + Med. 42: 506. 1939

90. Seyle, H. and L. Bassett. Proc. Soc. Exper. Biol. Med. 45:272. 1940

91. Kendall, E.C. J. Biol. Chem. Proc. 133: 11. 1940

The anabolic activity is produced by the anabolic factor and is mediated through the adrenal cortex.⁵⁵ In contrast to these results, Harrison and Harrison⁵⁶ reported that administration of desoxycorticosterone acetate prevents the fall of blood sugar in fasted animals. It may be that desoxycorticosterone influences anabolic activity indirectly through its effect on electrolyte balance. Bayle and Bennett⁵⁷, studying the effect of desoxycorticosterone on water and chloride metabolism in the infant rat, found a marked diuresis accompanied by an increased chloride excretion.

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55. Gratian, J. E. and E. L. Harrison. J. Biol. Chem. 1931 91: 1940

56. Harrison, H. E. and H. L. Harrison. J. Biol. Chem. 1931 91: 1940

57. Bayle, H. and J. Bennett. Proc. Soc. Exper. Biol. Med. 1932 25: 1940

58. Kendall, E. C. J. Biol. Chem. 1931 91: 1940

Table IV

RELATIVE QUANTITATIVE EFFECTS OF COMPOUNDS WHICH HAVE BEEN PROVEN TO
 REPLACE THE FUNCTIONAL ACTIVITY OF THE ADRENAL CORTEX WITH RESPECT TO
CARBOHYDRATE METABOLISM, MAINTENANCE OF LIFE, AND MUSCULAR EFFICIENCY

(Activity indicated as Marked, Moderate, or Slight)

Compound	Carbohydrate Metabolism	Maintenance of Life	Muscular Efficiency
17-Hydroxycorticosterone 17-Hydroxydehydrocorticosterone	Marked	Slight	Marked
Corticosterone 11-Dehydrocorticosterone	Moderate	Moderate	Moderate
Desoxycorticosterone Amorphous Fraction	Slight	Marked	Slight

B) PHYSIOLOGICAL ACTION OF THE CORTEX

Britton and Silvette⁹² were the first to emphasize the part played by the adrenal cortex in carbohydrate metabolism, and it was their paper which started the debate as to which was the dominant effect of the adrenal cortex.

The output of cortical hormone appears to be controlled, in part, by one of the secretions of the anterior pituitary body. This secretion has been called the adrenotropic factor of the pituitary, or simply, adrenotropin. Collip⁹³ and his associates have obtained an extract from the anterior pituitary which restores the atrophied adrenal cortex of the hypophysectomized animal to normal, and which is almost free from thyrotropic and gonadotropic effects. Bates, Riddle, and Miller⁹⁴, using a new method, have obtained the adrenotropic principle in greater concentration than heretofore achieved. Their preparation is essentially free from other pituitary factors. Russell⁹⁵, as well as Emery and Gottsch⁹⁶, have demonstrated that the life of adrenalectomized rats can be prolonged by pituitary extracts, or implants. Swann⁹⁷, reviewing

-
92. Britton, S.W. and H. Silvette. Am. J. Physiol. 100: 701. 1932
Cold Spring Harbor Symp. Quant. Biol. 5: 357. 1937
 93. Collip, J.B., E.M.Anderson, and D.L.Thomson. Lancet. 2: 347. 1933
 94. Bates, R.W., O. Riddle, and R.A.Miller. Endocrinology.
27: 781. 1940
 95. Russell, J.A. Am. J. Physiol. 128: 552. 1940
 96. Emery, F.E. and L.G.Gottsch. Endocrinology. 28: 321. 1941
 97. Swann, H.G. Physiol. Rev. 20: 493. 1940

the pituitary-adrenocortical relationship, stated flatly that adrenotropin controls the activity of the cortex in sugar, fat, and protein metabolism, to some extent its activities in protection of the organism against intoxication, and probably controls the adrenal's functions in muscle metabolism. He considers that the function of the adrenotropic factor of the pituitary is to preserve the lives and activities of the cells of the cortex, which, in turn, elaborate secretions important in the control of carbohydrate metabolism. That the anterior pituitary may exert its effects on carbohydrate metabolism by other means has already been suggested. Wyman and tum Suden⁹⁸ considered ". . . that the chief factor determining and limiting the growth of transplanted suprarenal cortical tissue is the available adrenotropic hormone of the host".

Long⁹⁹ concludes that a definition of the particular activity of the hormones of the anterior pituitary, e.g. adrenotropin, must rest on their isolation in pure form.

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98. Wyman, L.C. and C.tum Suden. Endocrinology. 21: 523. 1937
99. Long, C.N.H. Ann. Rev. Physiol. 4: 465. 1942

1) Effect on Absorption

A decrease in the absorption of glucose in adrenalectomized rats was reported by Cori and Cori¹⁰⁰ in 1927. Other evidence was offered in substantiation of these findings, and Verzar¹⁰¹ proposed the theory that in the absence of the adrenal cortical hormone, phosphorylation processes in the intestine are interfered with. These processes involved not only fat and vitamin B₂, but also carbohydrate. Several laboratories have tried to obtain confirmation of Verzar's results, but with little success. In fact, most of the data obtained tend to invalidate the theory. Houssay¹⁰² et al definitely showed that the rates of absorption of glucose, fructose, and xylose in the toad are not affected by adrenal insufficiency. Marrazzi¹⁰³, studying the comparative absorption of glucose and xylose in the adrenalectomized rat, found the absorption was decreased, but not to the same degree as reported by Verzar's group. She attributed the decreased absorption to other factors.

Clark and MacKay¹⁰⁴, working specifically on the problem of glucose absorption, concluded that the impaired glucose absorption in the adrenalectomized animal is not referable to the lack of influence of any adrenal cortical hormone on the intestinal mucosa but rather is consequent to the disturbance of salt metabolism.

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100. Cori, C.F. and G.T. Cori. J. Biol. Chem. 74: 473. 1927
101. Verzar, F. Ann. Rev. Biochem. 7: 163. 1938
102. Houssay, B.A., V.G. Foglia, and O. Fustinoni.
Endocrinology. 28: 915. 1941
103. Marrazzi, R. Am. J. Physiol. 131: 36. 1940
104. Clark, W.G. and E.M. MacKay. Am. J. Physiol. 137: 104. 1942

2) Effect on blood sugar

Britton and Silvette¹⁰⁵ have shown a reduction of 34% from the normal of the blood sugar in adrenalectomized rats. Long, Katzin, and Fry¹⁰⁶ have correlated this reduction with the period of starvation of the animal prior to the blood sugar determination, and find that the reduction is variable and dependant on the length of the starvation period. Thus, the sugar content of the blood fell from 97 mgms. per 100 cc. whole blood at the beginning of the fasting period, to a low of 30 mgms. after 48 hours of starvation. Evans¹⁰⁷ found a variation, but not quite in the same way. His values vary from 77 mgms. at the beginning, decreasing to 30 mgms. after 15 hours, and increasing to 53 mgms. at the end of 24 hours of fasting (values per 100 cc. whole blood).

Wyman and Walker¹⁰⁸ demonstrated in the completely adrenalectomized rat that the blood sugar values fall to between 50 and 60 mgms. per 100 cc. whole blood in the stage of subacute insufficiency, while in the terminal convulsive stages, it could reach a low value of 30 mgms. Their determinations were made on 24 hour fasting rats. The findings of these investigators are significant in that they indicate that the blood sugar varies not only with the length of the fasting period, but at least in part, varies with the degree of adrenal cortical insufficiency attained by the animal at the time of the sugar determination.

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105. Britton, S.W. and H.Silvette. Am. J. Physiol. 118: 594. 1937

106. Long, C.N.H., B. Katzin, and E.G.Fry. Endocrinology. 26: 309. 1940

107. Evans, G. Endocrinology. 29: 731. 1941

108. Wyman, L.C. and B.S.Walker. Am. J. Physiol. 89: 215. 1929

These factors, length of fasting period and degree of adrenal insufficiency of the experimental animal, may explain the variation in blood sugar values reported by different laboratories. Other factors are probably involved. As was previously pointed out, the conditions existent in the laboratory and the techniques of the procedures employed will affect the results. Evans¹⁰⁹ suggested that in glucose feeding tests, not only is the condition of the animal important, but also the dose of glucose.

It is apparent, however, that there is an evident reduction from the normal of the blood glucose in adrenalectomized animals.

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109. Evans, G. Endocrinology. 29: 731. 1941

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also the dose of glucose.

It is apparent, however, that there is an evident reduction
from the normal of the blood glucose in adrenalectomized animals.

3) Effect on liver glycogen

Long and his associates¹¹⁰ have shown that the liver glycogen is reduced in adrenalectomized rats. Their results indicated that the level of liver glycogen remained normal as long as the animal is fed. However, on fasting, there was a rapid decline in the liver glycogen, which fell to a low point of 16 mgms (per 100 gms. wet liver) after 24 hours, to be followed by a rise to 70 mgms. after 48 hours. They further found that an administration of a cortical extract to adrenalectomized rats following a preliminary fasting period of 24 hours served to increase the glycogen stores of the liver during the 24 hours of fasting immediately following. They postulated that "... one of the properties of the cortical hormone is a stimulation of protein catabolism, and that the increased carbohydrate levels . . . is an expression of this effect."

Sprague¹¹¹ observed that an extract of the adrenal cortex produced an increase in the amount of glycogen stored in the liver of fasting normal rats. Thus, after 24 hours, the untreated rats had a liver glycogen of 320 mgms (per 100 gms. wet liver), while those treated with cortical extract had a liver glycogen of 1470 mgms. These experiments gave results similar to those reported by Katzin and Long¹¹².

110. Long, C.N.H., B. Katzin, and E.G.Fry. Endocrinology. 26: 309. 1940

111. Sprague, R.G. Proc. Staff Meet. Mayo Clin. 15: 291. 1940

112. Katzin, B. and C.N.H.Long. Am. J. Physiol. 123: P113. 1938

A lowered liver glycogen in the adrenalectomized rat has been found by Evans¹¹³, Russell¹¹⁴, and Buell et al¹¹⁵.

The fact seems to be well established that in the adrenalectomized animal the ability to maintain the liver glycogen stores is impaired.

-
113. Evans, G. Endocrinology. 29: 731. 1941
114. Russell, J. Am. J. Physiol. 128: 552. 1940
115. Buell, M.V., I.A. Anderson, and M.B. Strauss.
Am. J. Physiol. 116: 274. 1936

4) Effect on muscle glycogen

Numerous investigators have shown the muscle glycogen to fall below normal in the adrenalectomized rat. Britton¹¹⁶ obtained a fall of 51% from the normal for muscle glycogen in adrenalectomized rats. Long¹¹⁷ and his associates obtained a muscle glycogen lowered from the normal in such animals, with the extent of lowering dependent on the length of the fasting period which the animal had undergone.

However, Sprague¹¹⁸, as well as Katzin and Long¹¹⁹, found no significant increase in the muscle glycogen of normal animals following administration of adrenal cortical extracts. Abelin and Althaus¹²⁰ believed that adrenal cortical extract has no effect on the glycogen metabolism of muscle. Long concluded¹²¹ :

"At the present time, therefore, there is no evidence that the cortical hormones that influence carbohydrate metabolism may do so (a) by inhibiting the peripheral utilization of glucose, (b) by inhibiting liver glycogenolysis or accelerating the conversion of glucose to glycogen, (c) by controlling the rate of deamination of amino acids and in consequence the rate of glyconeogenesis from protein."

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- 116. Britton, S.W. and H. Silvette. *Am. J. Physiol.* 118: 594. 1937
 - 117. Long, C.N.H., B. Katzin, and E.G.Fry. *Endocrinology*. 26: 309. 1940
 - 118. Sprague, R.G. *Proc. Staff Meet. Mayo Clin.* 15: 291. 1940
 - 119. Katzin, B. and C.N.H.Long. *Am. J. Physiol.* 123: P113. 1938
 - 120. Abelin, I. and U. Althaus. *Helv. Chim. Acta.* 25: 205. 1942
 - 121. Long, C.N.H. *Ann. Rev. Physiol.* 4: 465. 1942

Fraenkel-Conrat et al¹²² have studied the effects of adrenalectomy on liver arginase and suggest that the effect of the adrenal cortical hormone on certain enzyme systems is basically responsible for the role of the adrenal cortex in the control of carbohydrate metabolism.

In an excellent paper, Ingle¹²³ has summarized the principle factors involved in carbohydrate metabolism and discussed their relationships and problems involved in adrenal cortical insufficiency. The emphasis is placed on the unsettled problems.

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122. Fraenkel-Conrat, H., M.E.Simpson, and H.M.Evans.
J. Biol. Chem. 147: 99. 1943

123. Ingle, D.W. Endocrinology. 31: 419. 1942

VI. RELATIVE INSUFFICIENCY OF ADRENAL CORTICAL HORMONE

Following the demonstration by Jaffe and Plavska¹²⁴, ¹²⁵ that the adrenal cortex could be transplanted successfully in rats and guinea pigs, it was assumed that autoplasmic cortical transplants in adrenalectomized rats fully replaces the functions of the excised gland. That this is not altogether true has since been shown by the results of many experiments.

The most significant studies of the physiology of transplanted adrenal cortical tissue have been made by Wyman and tum Suden. These investigators have been engaged for more than a decade in investigations concerning the function of the adrenal cortex, and have published a long list of papers demonstrating the efficiency of the transplanted tissue. In many instances, the adrenalectomized rat with autoplasmic cortical transplants was shown to be like the normal rat; in others, it was shown to be intermediate to the normal and completely adrenalectomized rat.

Since this paper deals with carbohydrate metabolism in the rat with adrenocortical transplants, a survey of some of the previous work performed in their (Wyman and tum Suden's) laboratory does not seem unwarranted.

In 1926, Belding and Wyman¹²⁶ demonstrated that adrenalectomized rats having autoplasmic cortical transplants (hereinafter called 'transplants') were like normal rats with respect to diphtheria toxin, but

124. Jaffe, H.L. and A.Plavska. Proc. Soc. Exper. Biol. Med.

28: 528. 1926

125. Jaffe, H.L. Amer. J. Path. 2: 421. 1926

126. Belding, D.L. and L.C.Wyman. Am. J. Physiol. 78: 50. 1926

that adrenalectomized rats are more susceptible to the toxin than is the normal rat. In this same paper, a careful description was given of the selection of the animals for experimentation, care of the animal room, diet, after-care, and the technique of adrenalectomy and control operations.

Wyman¹²⁷ in 1928 reported that the adrenalectomized rat suffers a disturbance of ovarian function, the degree of which is correlated with the degree of insufficiency. He suggested that ". . . the relationship between the functioning of the suprarenal glands and the gonads is not direct but that it is indirect through the mediation of other factors, probably metabolic in nature". Thus Wyman anticipated the connection between the ovarian and cortical hormones (both of which are steroids).

In the same year, Wyman¹²⁸ reported that the adrenalectomized rat was extremely susceptible to small doses of histamine, and confirmed the results of Crivellari¹²⁹, Marmorston-Gottesman and Gottesman¹³⁰, and Scott¹³¹. He also observed that the 'transplant' was quite as susceptible to histamine poisoning as the adrenalectomized rat. Here then was offered noteworthy evidence that the 'transplant' was not normal in all respects. Later experimentation produced similar results (Wyman and tum Suden)¹³². These effects were considered to be due to a lack of medullary tissue rather than to a lack of cortical tissue. Contrary

127. Wyman, L.C. Am. J. Physiol. 86: 528. 1928

128. Wyman, L.C. Am. J. Physiol. 87: 29. 1928

129. Crivellari, C.A. Am. J. Physiol. 86: 414. 1927

130. Marmorston-Gottesman, J. and J. Gottesman.
J. Exper. Med. 47: 503. 1928

131. Scott, W.J.M. J. Exper. Med. 47: 185. 1928

132. Wyman, L.C. and C. tum Suden. Am. J. Physiol. 99: 285. 1932

to this opinion, Perla and Marmorston¹³³ offered evidence that the increased susceptibility to histamine was due to a loss of cortical hormone.

In 1937, Wyman and tum Suden¹³⁴ concluded that the difference in strain, mixed colored and hooded varieties vs. inbred pure albino, had been a major factor responsible in their earlier work for the relative insufficiency of the transplanted tissue in histamine shock, and offered new evidence suggesting that the susceptibility varied with the amount of cortical secretion available. They found that adrenalectomized rats were most susceptible, 'transplants' were less so, 'transplants' plus administration of a cortical extract even less susceptible, and the normal rat the least. With these results, they concluded that the increased susceptibility of the rat to histamine after adrenalectomy depends both on medullary and cortical lack.

The blood sugar, non-protein nitrogen and urea nitrogen levels of the 'transplant' were shown to be like that of the normal by Wyman and Walker^{135, 136} in 1929. In the adrenalectomized rat, their results confirmed other investigations; that the blood sugar was markedly depleted, and the non-protein and urea nitrogen increased, the amount of depletion or increase paralleling the severity of the symptoms of adrenal insufficiency. Concerning the blood sugar level, they concluded the medulla was not essential for its maintenance, and suggested that the cortex played a part in the 'steady maintenance' of that level.

133. Perla, D. and J. Marmorston-Gottesman. Proc. Soc. Exper. Biol. Med. 28: 650. 1931

134. Wyman, L.C. and C. tum Suden. Endocrinology. 21: 587. 1937

135. Wyman, L.C. and B.S. Walker. Am. J. Physiol. 89: 215. 1929

136. Wyman, L.C. and B.S. Walker. Am. J. Physiol. 89: 349. 1921

Wyman and tum Suden¹³⁷ correlated blood sugar levels with histamine shock, and found that following injection of small doses of histamine in normal rats, a hyperglycemia appeared. This they ascribed to adrenal medullary effects. Injection of comparable doses in either the adrenalectomized rat or the 'transplant' resulted in hypoglycemia. These results convinced them that adrenal insufficiency could still be spoken of as a "shock-like condition".

That the presence of the adrenal cortex is necessary for temperature regulation in the rat was shown in 1929¹³⁸ and further evidence supporting this was offered in 1932¹³⁹.

Blood volume in the transplant was demonstrated to be like that of the normal rat (Wyman and tum Suden)¹⁴⁰.

Several studies of the factors determining growth and efficiency of transplanted adrenal cortical tissue indicated that the total amount of cortical tissue regenerating in the 'transplant' is fairly constant for each sex, irrespective of the number of transplanted fragments present¹⁴¹; that the adrenotropic hormone of the anterior pituitary is necessary for such growth¹⁴²; and that transplanted cortical tissue does not function as efficiently as does the normal gland in acute

137. Wyman, L.C. and C. tum Suden. Am. J. Physiol. 108: 424. 1934

138. Wyman, L.C. and C. tum Suden. Am. J. Physiol. 89: 362. 1929

139. Wyman, L.C. and C. tum Suden. Proc. Soc. Exper. Biol. Med. 30: 61. 1932

140. Wyman, L.C. and C. tum Suden. Am. J. Physiol. 94: 579. 1930

141. Wyman, L.C. and C. tum Suden. Am. J. Physiol. 101: 662. 1932

142. Wyman, L.C. and C. tum Suden. Endocrinology. 21: 523. 1937

emergencies, e.g. histamine poisoning. It was suggested that the efficiency of the tissue in this respect varies both individually and with colony¹⁴³. Recently reported data have confirmed these earlier observations that¹⁴⁴

"... in the rat, intramuscularly transplanted adrenocortical tissue, although able to maintain life and general health, is less efficient than the normal cortex in acute emergencies, especially those which demand the aid of additional cortical hormone in producing prompt redistribution of body fluids. The efficiency of transplanted tissue also varies among individuals."

Mention has been made that adrenal insufficiency may be spoken of as a "shock-like" condition. Wyman¹⁴⁵ early noted the similarity between histamine reactions and anaphylactic shock phenomena, and extended investigations of these effects to the 'transplant'. He found such animals to be as susceptible to anaphylactic shock as the adrenalectomized rat. Here was added evidence that the 'transplant' was unlike the normal rat in certain respects. In 1930, Wyman and tum Suden¹⁴⁶ studied the blood volume in rats after anaphylactic and histamine shock, and found it to be reduced from the normal. They suggested that

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143. Wyman, L.C. and C. tum Suden. Endocrinology. 21: 587. 1937

144. Wyman, L.C. and C. tum Suden. Endocrinology. 31: 295. 1942

145. Wyman, L.C. Am. J. Physiol. 89: 356. 1929

146. Wyman, L.C. and C. tum Suden. Am. J. Physiol. 94: 579. 1930

lack of medullary adrenal secretion was responsible for the animal's inability to maintain capillary tone and normal permeability. In a reanalysis¹⁴⁷ of their previous data, together with additional experimental results, they have recently been led to conclude that the inability of the 'transplant' to combat anaphylactic shock is due largely to some inefficiency in cortical function, and to a lesser degree to a lack of medullary support, and that the 'transplant', under conditions of stress, lies intermediate to the normal and the adrenalectomized animal, even though the amount of regenerated tissue is comparable to that in both intact adrenals.

In the foregoing pages, it has been pointed out that a condition of lowered glycogen content of the liver and muscles exists in completely adrenalectomized rats. In common with other investigators, Wyman and Walker¹⁴⁸ found that the blood sugar suffered a marked drop in completely adrenalectomized rats, but no significant variation from the normal was found in adrenalectomized rats having autoplasmic cortical transplants.

It seemed pertinent, therefore, to further these investigations by making an analysis of the quantitative relationships of the blood sugar (1), the liver glycogen (2), and the muscle glycogen (3) in adrenalectomized animals having autoplasmic cortical transplants. The experiments to be described in the following pages present such an analysis.

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147. tum Suden, C. and L.C.Wyman. Personal communication

148. Wyman, L.C. and B.S.Walker. Am. J. Physiol. 89: 215. 1929

VII. EXPERIMENTAL PROCEDURES

A) THE EXPERIMENTAL ANIMAL

The rats used in this investigation were obtained from a stock colony maintained at the Boston University School of Medicine. This colony consisted of a mixed stock of albino rats, which had shown itself to be fairly resistant to the effects of removal and transplantation of the adrenal gland. The animals received a diet of Purina dog chow, supplemented with greens. No attempt was made to keep the animals in separate cages, which were kept in the animal room, free from drafts, and at a comfortable temperature.

Both adrenal glands were removed in one operation by the posterior route. (The operations were performed by Dr. Caroline tum Suden). Each gland was divided in half and four implants made: two anteriorly, one on each side, by inserting a half gland in the shoulder muscles; and two posteriorly, at the border of the inguinal fat, one on each side. On completion of the operation, the animal was placed in a cage with other 'operated animals'. Post-operative care consisted of keeping the rats protected from drafts. Otherwise the treatment was the same as given to the normal animal. Recovery was uneventful in most cases, and resort to saline drinking water was not necessary. At the time each animal was killed (for the chemical determinations), examination was made of each implantation site, as well as a wide area surrounding the original site of the gland, for evidence of hypertrophy, rests, and fragments.

B) THE FASTING STATE

Before the beginning of the fasting period, the animals were removed from the animal room and placed in cages in the laboratory where the procedures for the chemical determinations were to be carried out. The animals were permitted access to food up to the beginning of the period. At a given hour, usually in the morning, the food was removed from the cage, the time noted and the period of fasting begun. At all times water was available to the animals. At the end of the 24 hour fasting period, determinations on two groups of rats were made of the blood glucose, glycogen in the liver, and glycogen in the muscle; adrenalectomized rats with transplants composed one group; control animals, the other.

C) THE GLUCOSE MEAL

On other groups (three, control animals; three, animals with transplanted adrenals; and one, adrenalectomized animals) a glucose meal was given after the 24 hours of fasting. The amount of glucose given depended on the body weight (1.065 gms. of glucose per 100 gms. of body weight), and was administered by stomach tube, in the following manner. Handling the animal gently, to avoid excitement, the head was bent slightly backwards. This was accomplished by holding the animal by the scruff of the neck, and exerting a slight pull. Then a small catheter tube, the end of which had been dipped into the glucose solution, was inserted into the side of the animal's mouth, and, with a gentle pressure, was guided down its oesophagus into the stomach. A graduated syringe containing the sugar solution was attached to the other end of the tube, and the required amount of glucose very slowly passed into the stomach. The tube was then quickly withdrawn and the animal placed back in its cage, the time being noted in each case.

D) SAMPLING

At the proper time (1,2,etc. hours after the glucose meal), the rat was anesthetized with amytal (intraperitoneal injection) (10 mgms. per 100 gms. body weight). The abdominal cavity was then opened and samples removed in the following order. Blood was obtained from the vena cava, and placed immediately in an oxalated bottle (lithium oxalate). The syringe used for obtaining the blood had first been wetted with heparin solution. In most cases, blood was obtained before coagulation set in. A number of specimens had to be discarded, however, because the blood coagulated too quickly or showed evidence of a slight amount of coagulation.

The whole liver was then carefully dissected out, and removed to a piece of paper toweling. Any adherent blood was removed by pressing the liver gently against the toweling and permitting the blood to soak through. The liver was then weighed and placed immediately in hot 30 % potassium hydroxide solution.

Samples of muscles were then obtained by removing the gastrocnemius from one or both legs. Immediately on removal, the muscle tissue was weighed and placed in hot 30% KOH.

The whole of this procedure, from the injection of the anesthetic to the placing of the final sample (muscle) in KOH took approximately 15 minutes.

Subsequent to these procedures, the animal was examined for evidence as to the growth of the adrenocortical transplants.

E) BLOOD SUGAR

To one volume of blood, seven volumes of water were added. One volume of 10% solution of zinc sulfate was then added and the solutions mixed. With continuous shaking, one volume of 0.5 N sodium hydroxide was then added. The flask was then stoppered, thoroughly shaken, and the contents filtered, after a few minutes, through dry filter paper. Glucose was finally determined on the filtrate.

F) TISSUE GLYCOGEN

Liver and muscle glycogen were determined in precisely the same way and their determination will be described as one procedure.

Immediately following weighing, the tissue (liver or muscle) was placed in a 25 x 225 mm. tube containing hot 30% potassium hydroxide solution (2 cc. per gram. of tissue), and kept hot until a homogeneous solution had been formed (a matter of 10-20 minutes). Cori¹⁴⁹ found that KOH is as efficacious at room temperature as it is hot, but to avoid the possibility of any enzymatic destruction of the glycogen, heat was used here.

The glycogen was precipitated out of the hot solution by adding 1.1 to 1.2 volumes of 95% alcohol. The material was then centrifuged and the supernatant poured off. The glycogen was then dissolved in water and re-precipitated with alcohol, and the supernatant poured off. Following this, 1 N sulfuric acid was added to the glycogen precipitate in the tube and the whole made up to known volume. On the top of the tube was placed an air condenser. The glycogen in the sulfuric acid was then hydrolyzed by heating, in a boiling water bath, for a period of three and one-half to four hours. Up to this point, it is to be noted that the whole procedure was carried out in the same tube, thereby preventing errors arising from transfer procedures.

After hydrolysis was complete, an aliquot was removed from the tube, and neutralized with sodium hydroxide (using phenol red as an indicator, This was then made up to a known volume. To five cc. of

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149. Cori, C.F. and G.T. Cori. J. Biol. Chem. 100: 323. 1933

the neutralized hydrolysate, 5 cc. of the Shaffer-Hartman reagent was added, and the glucose determined in the usual manner.

The details of the preparation of the glucose solution, and the determination of blood glucose, liver and muscle glycogen are presented in appendices A to C.

The results of the determinations are presented in Tables V to X.

Figures 7, 8, and 9 graphically portray the results.

TABLE V

CARBOHYDRATE LEVELS IN NORMAL MALE RATS, AFTER 24 HOURS OF FASTING

Number	Body Weight in Gms.	Liver Weight in Gms.	Muscle Weight in Gms.	Blood Glucose ²	Liver Glycogen ³	Muscle Glycogen ⁴
N103	210	7.080	2.363	67	8	279
N102	207	6.953	2.743	66	23	304
NB	254	7.788	2.767	84	24	388
NT	221	7.016	2.540	76	27	433
N105	176	6.203	2.187	93	28	243
N104	207	7.073	2.340	70	30	343
N101	200	7.628	2.453	102 ¹	83	240
NX	279	7.368	3.270	82	146	346
Aver.	219	7.139	2.583	77	32	323

1. Not included in obtaining average results

2. Mgms. per 100 cc. whole blood

3. Mgms. per 100 gms. wet liver

4. Mgms. per 100 gms. wet muscle

TABLE VI

CARBOHYDRATE LEVELS IN MALE RATS WITH ADRENOCORTICAL TRANSPLANTS

AFTER A 24 HOUR FASTING PERIOD

Number Number	Body Weight in Gms.	Liver Weight in Gms.	Muscle Weight in Gms.	Blood Glucose ²	Liver Glycogen ³	Muscle Glycogen ⁴
T300a	176	5.823	1.002	105 ¹	4	542
T149	248	7.512	1.415	92	12	460
T246	220	7.090	2.065	83	17	114 ¹
T43	254	8.774	2.842	62	20	479
T41-5	273	8.208	3.050	64	22	400
R300B	164	5.322	0.950	103 ¹	26	489
T41-40	307	9.161	3.862	76	30	451
T133	284	7.355	1.137	103 ¹	31	438
T88	274	8.535	3.367	111 ¹	36	568
T41-1	300	8.720	3.050	94	42	444
T41-3	271	7.980	3.076	64	50	415
T41-4	302	9.230	3.470	79	52	448
T90	290	8.168	3.403	91	55	591
T141	380	11.020	2.065	87	91	512
T91	276	8.815	3.325	88	109 ¹	567
T41-45	238	7.614	2.840	100 ¹	297 ¹	459
T135	292	8.735	1.568	149 ¹	308 ¹	214 ¹
Ave.	268	8.121	2.499	80	35	484

1. Not included in obtaining the average results.

2. Mgms. per 100 cc. whole blood

3. Mgms. per 100 gms. wet liver

4. Mgms. per 100 gms. wet muscle.

TABLE VII

CARBOHYDRATE LEVELS IN NORMAL MALE RATS AFTER A 24 HOUR FASTING PERIOD
THEN GIVEN A GLUCOSE MEAL. ANIMALS KILLED AT VARYING INTERVALS.

Number	Body Weight in Gms.	Liver Weight in Gms.	Muscle Weight in Gms.	Hours After Meal	Blood Glucose ¹	Liver Glycogen ²	Muscle Glycogen ³
Series A.							
N1	201	6.115	2.570	2.0	84	525	424
N2	208	6.385	2.630	2.3	110	254	211
N3	258	7.860	2.850	2.3	92	1340	454
N4	281	8.565	2.890	2.6	124	777	591
N5	172	5.180	2.095	2.6	127	1102	500
N6	230	7.905	2.685	3.2	122	2400	519
Series B.							
NB	201	6.090	2.560	1.0	152	426	226
NE	203	6.330	2.520	2.0	96	961	322
NA	232	7.710	2.825	3.0	129	1280	103
ND	204	6.625	2.645	4.0	148	2960	549
NC	150	6.110	1.800	5.0	140	3650	386
Series C.							
NS	229	6.980	2.885	1.0	85	282	392
TS	214	6.815	2.580	2.0	94	1679	471
BS	192	6.815	2.145	3.0	142	1702	635
BX	176	6.135	2.035	4.0	162	3116	605
AW	170	5.860	1.845	5.0	151	3830	706
H	159	5.985	1.740	6.0	108	4800	632
HT	155	6.470	1.845	7.0	143	5170	895

1. Mgms. per 100 cc. whole blood

2. Mgms. per 100 gms. wet liver

3. Mgms. per 100 gms. wet muscle.

TABLE VIII

CARBOHYDRATE LEVELS IN MALE RATS WITH ADRENOCORTICAL TRANSPLANTS.
 AFTER A 24 HOUR FASTING PERIOD. THEN GIVEN A GLUCOSE MEAL.
 ANIMALS KILLED AT VARYING INTERVALS.

Number	Body Weight in Gms.	Liver Weight in Gms.	Muscle Weight in Gms.	Hours After Meal	Blood Glucose ¹	Liver Glycogen ²	Muscle Glycogen ³
Series A,							
42-10	283	8.360	3.665	1.0	118	334	442
42-11	252	8.060	2.935	2.0	114	1150	503
42-76	262	8.370	3.115	2.5	80	48	447
42-24	241	8.635	2.645	3.0	90	1276	513
45-20	203	7.230	2.570	4.0	113	1840	506
Series B.							
58	214	7.580	2.095	1.0	106	275	194
62	233	7.635	2.905	2.0	116	399	279
59	224	7.810	2.585	3.0	138	890	448
61	192	6.760	5.495	4.0	156	1285	177
54	206	7.790	2.500	5.0	118	2720	417
Series C.							
73	245	7.690	2.480	1.0	88	223	490
74	244	8.205	2.725	2.0	128	35	456
75	244	8.910	2.905	3.0	116	2077	618
78	213	7.860	2.560	4.0	127	2795	667
76	202	7.980	2.390	5.0	136	3040	655
80 ⁴	186	7.325	2.255	6.0	---	990	386
83	174	7.620	2.090	7.0	108	3610	526

1. Mgms. per 100 cc. whole blood

2. Mgms. per 100 gms. wet liver

3. Mgms. per 100 gms. wet muscle

4. Animal '80' was suffering from an acute insufficiency of adrenal cortical hormone. This was evident from the animal's appearance, the difficulty in obtaining blood, and the results of the chemical determinations.

TABLE IX

CARBOHYDRATE LEVELS IN COMPLETELY ADRENALECTOMIZED MALE RATS⁴, THREE DAYS AFTER OPERATION. AFTER A 24 HOUR FASTING PERIOD. THEN GIVEN A GLUCOSE MEAL. ANIMAL KILLED AT VARYING INTERVALS.

Number	Body Weight in Gms.	Liver Weight in Gms.	Muscle Weight in Gms.	Hours After Meal	Blood Glucose ¹	Liver Glycogen ²	Muscle Glycogen ³
A	221	7.270	2.680	1.0	---	44	286
B	200	6.380	2.515	2.0	---	48	289
C	210	6.415	2.505	3.0	---	55	475
D	231	7.135	2.840	4.0	---	30	362
E	226	6.770	2.725	5.0	---	37	494

1. Mgms. per 100 cc. whole blood

2. Mgms. per 100 gms. wet liver.

3. Mgms. per 100 gms. wet muscle.

4. These animals were very sick, apathetic, listless, and oblivious of surroundings. Their blood was thick and viscous, and samples were not obtainable by the methods used (i.e. obtaining blood via the vena cava). Several animals of this group died before the 24 hour fasting period was completed. Several others died shortly after being given the glucose meal, and could not be used for the chemical determinations.

TABLE X

AVERAGE VALUES¹ (COMBINING TABLES V, VI, VII, VIII, IX) FOR CARBOHYDRATE LEVELS OF THE BLOOD, LIVER, AND MUSCLE IN COMPLETELY ADRENALECTOMIZED RATS (A), ADRENALECTOMIZED RATS WITH AUTOPLASTIC TRANSPLANTS (T), AND IN RATS THAT ARE NORMAL (N).

	Blood Glucose ²				Liver Glycogen ³				Muscle Glycogen ⁴		
	A	N	T		A	N	T		A	N	T
After 24 hour fast	---	77 ₇	80 ₁₁		---	32 ₇	35 ₁₄		---	323 ₈	484 ₁₅
Hours After Meal	Values after glucose meal										
1.0	---	119 ₂	104 ₃		44 ₁	354 ₂	277 ₃		286 ₁	309 ₂	375 ₃
2.0	---	91 ₃	119 ₃		216 ₁	1055 ₃	528 ₃		289 ₁	406 ₃	413 ₃
3.0	---	101 ₂	---		---	787 ₂	---		---	333 ₂	---
2.5	---	---	80 ₁		---	---	48 ₁		---	---	447 ₁
2.6	---	126 ₂	---		---	940 ₂	---		---	546 ₂	---
3.0	---	136 ₂	115 ₃		55 ₁	1491 ₂	1414 ₃		475 ₁	369 ₂	526 ₃
3.2	---	122 ₁	---		---	2400 ₁	---		---	519 ₁	---
4.0	---	155 ₂	132 ₃		30 ₁	3038 ₂	1973 ₃		362 ₁	577 ₂	450 ₃
5.0	---	146 ₂	127 ₂		37 ₁	3740 ₂	2880 ₂		494 ₁	546 ₂	536 ₂
6.0	---	108 ₁	---		---	4800 ₁	---		---	632 ₁	386 ₁
7.0	---	143 ₁	108 ₁		---	5170 ₁	3610 ₁		---	895 ₁	526 ₁

1. Sub-scripts refer to number of cases used to obtain averages.

2. Mgms. per 100 cc. whole blood.

3. Mgms. per 100 gms. wet liver

4. Mgms. per 100 gms. wet muscle

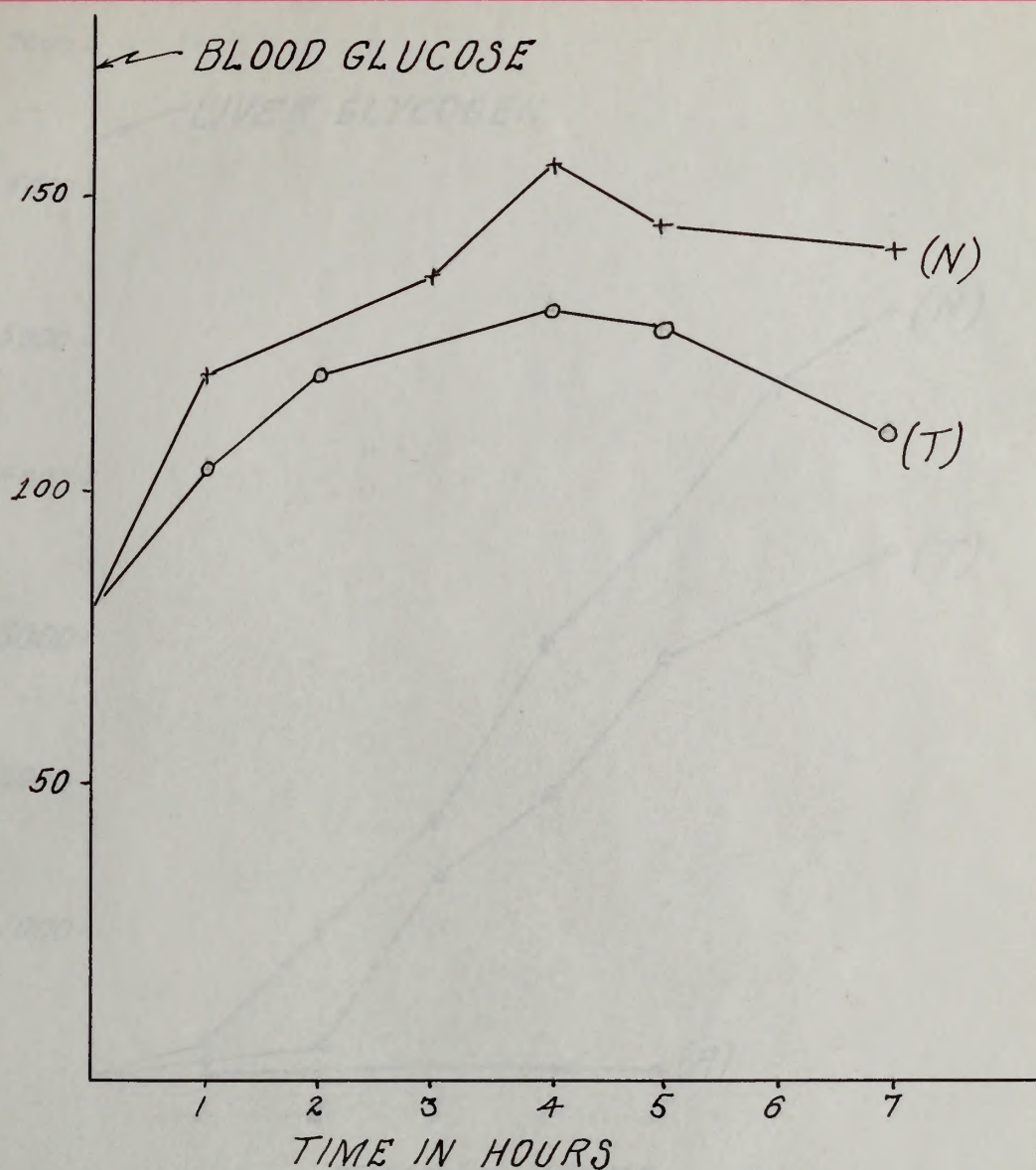


FIG. 7. CHANGES IN BLOOD GLUCOSE (MGMS.)
PER 100 cc. WHOLE BLOOD) AFTER A GLUCOSE MEAL
NORMAL RAT = N 'TRANSPLANT' RAT = T

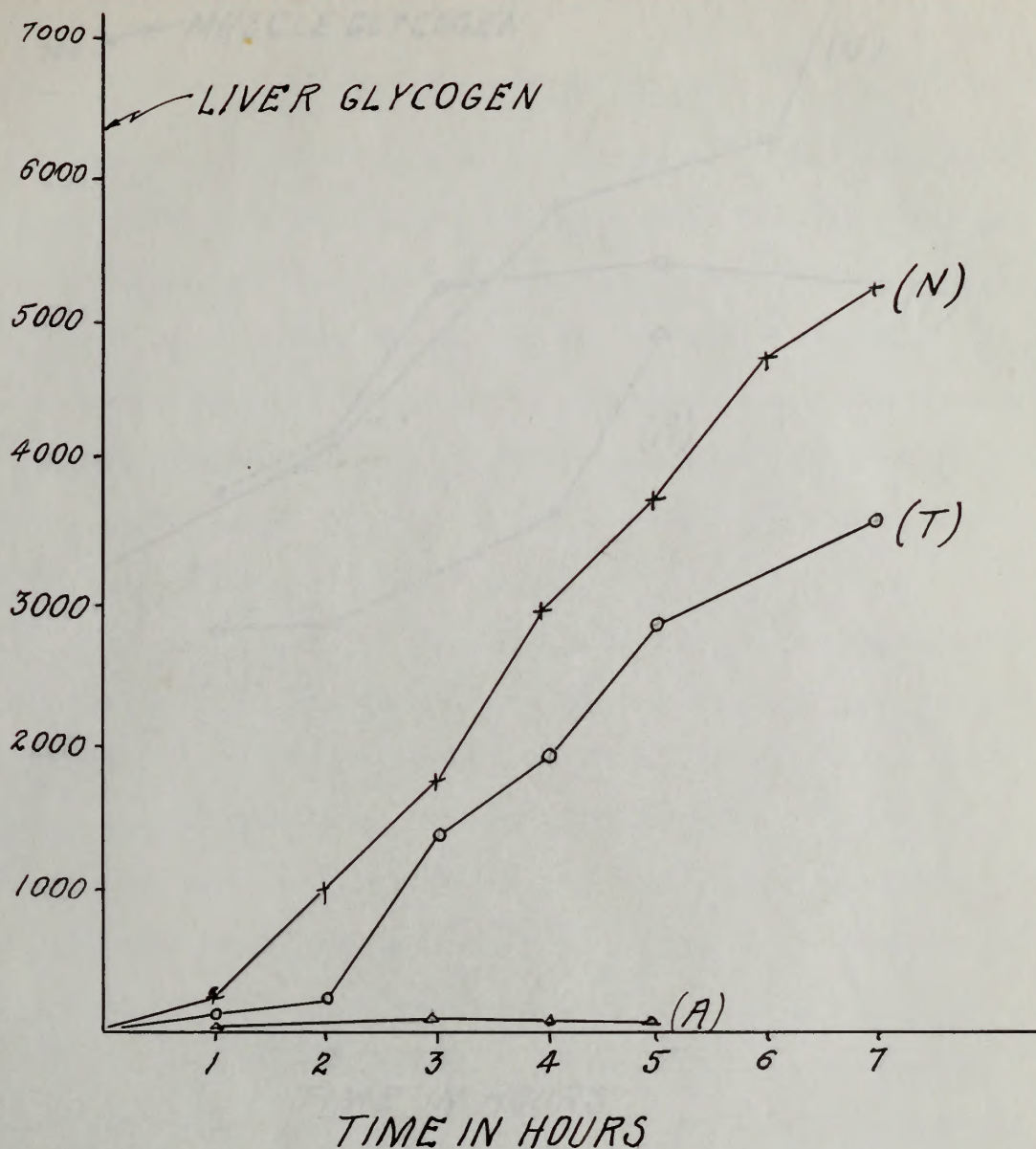


FIG. 8. CHANGES IN LIVER GLYCOGEN (MGMS. PER 100 GMS. WET LIVER) FOLLOWING A GLUCOSE MEAL IN THE NORMAL RAT (N), RAT WITH ADRENOCORTICAL TRANSPLANTS (T), AND ADRENALECTOMIZED RAT (A).

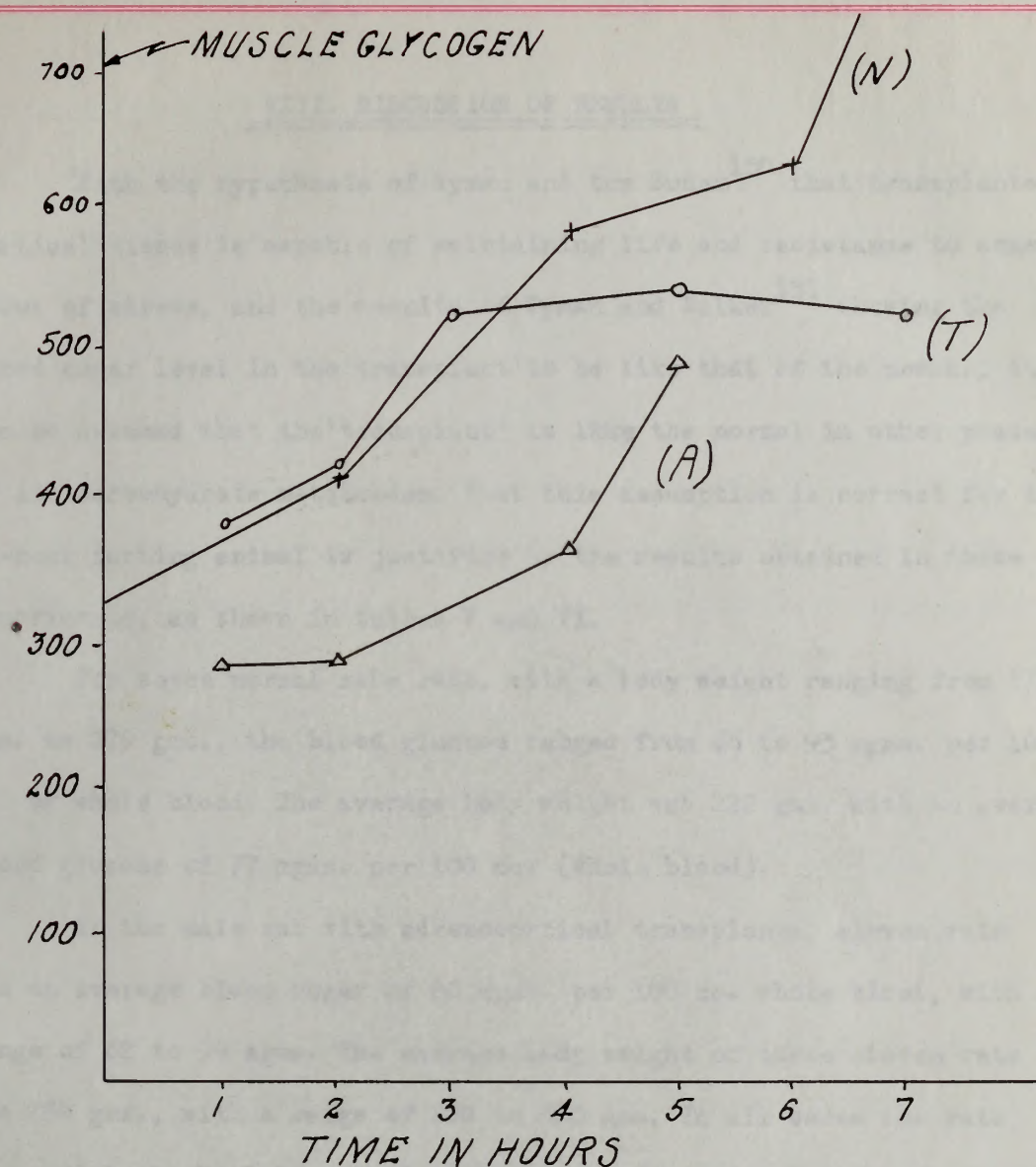


FIG. 9. CHANGES IN MUSCLE GLYCOGEN
(MGMS. PER 100 GMS. WET MUSCLE). NORMAL RAT = N
RAT WITH ADRENOCORTICAL TRANSPLANTS = T
ADRENALECTOMIZED RAT = A

VIII. DISCUSSION OF RESULTS

With the hypothesis of Wyman and tum Suden¹⁵⁰ that transplanted cortical tissue is capable of maintaining life and resistance to some forms of stress, and the results of Wyman and Walker¹⁵¹ showing the blood sugar level in the 'transplant' to be like that of the normal, it can be assumed that the 'transplant' is like the normal in other phases of its carbohydrate metabolism. That this assumption is correct for the 24-hour fasting animal is justified by the results obtained in these experiments, as shown in tables V and VI.

For seven normal male rats, with a body weight ranging from 176 gms. to 279 gms., the blood glucose ranged from 66 to 93 mgms. per 100 cc. of whole blood. The average body weight was 222 gms. with an average blood glucose of 77 mgms. per 100 cc. (whole blood).

In the male rat with adrenocortical transplants, eleven rats had an average blood sugar of 80 mgms. per 100 cc. whole blood, with a range of 62 to 94 mgms. The average body weight of these eleven rats was 284 gms., with a range of 220 to 380 gms. In all cases the rats appeared to be healthy and at autopsy were found to have abundant body fat.

These values are well within the range of values obtained by Wyman and Walker¹⁵¹. (Their average blood sugar value was 82 mgms. per 100 cc. whole blood for the normal rat).

150. Wyman, L.C. and C. tum Suden. Endocrinology. 21: 587. 1937

151. Wyman, L.C. and B.S. Walker. Am. J. Physiol. 89: 215. 1929

Since Somogyi's¹⁵² procedure for the precipitation of blood proteins was used in these experiments, the results obtained here are probably nearer the "true" blood sugar than the results of Wyman and Walker. However, there results quite substantiate their claim that the rat with adrenaocortical transplants does not vary significantly from the normal with respect to its blood sugar.

The liver glycogen at the end of the 24 hour fasting period was 32 mgms. per 100 gms. liver for seven normal male rats, and 35 mgms. per 100 gms. liver for fourteen rats with adrenocortical transplants. Ranges for the normal animal were 8 - 83 mgms.; for the transplant, 4 - 91 mgms.

The values of the liver glycogen obtained are in agreement with those reported by other (see table I), and it is concluded that the rat with adrenocortical transplants is like the normal with respect to its liver glycogen, at the end of a 24 hour fasting period.

Values for muscle glycogen ranged from 240 - 433 mgms. per 100 gms. wet muscle, averaging 323 mgms. in the normal male rat (eight cases), while in a larger number of cases (fifteen) of males with transplants, the muscle glycogen was 484 mgms. per 100 gms. muscle, with a range of 400 - 591 mgms. These values are considered to be normal, even though slightly lower than the values reported in table II. (The normal animals in this group were in a poorer physical condition than the 'transplants', having been subjected to a difficultly controlled parasitic infestation previous to the chemical determinations).

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152. Somogyi, M. J. Biol. Chem. 86: 655. 1930

The results give evidence that with respect to muscle glycogen, the 'transplant' is not unlike the normal after 24 hours of fasting.

These data justify the conclusion that the adrenalectomized rat with transplanted adrenal cortical tissue is like the normal rat after a 24 hour period of fasting with respect to its blood glucose, liver glycogen, and muscle glycogen.

The question arises: does transplanted tissue react as efficiently in the reverse condition, that is, when the animal is provided with an excess of carbohydrate?

Evans¹⁵³ has demonstrated that the "adrenalectomized animal provided with a carbohydrate plethora stores less and oxidizes more carbohydrate than does the intact animal". He observed that as far as return to original levels was concerned, the adrenalectomized animals have a normal glucose tolerance. In his experiments, the glucose was administered intravenously.

In examining the curves shown in figures 7, 8, and 9, the following points may be noted. In figure 7, (a) the glucose tolerance of the 'transplant' is like the normal with respect to the shape of the curve, but (b) is less sharp and has slightly lower values than those obtained for the normal animal. Figure 8: (a) the relative failure of the adrenalectomized rat to deposit glycogen is definite and is in agreement with other reports¹⁵³, (b) the normal rat is able to absorb and store the glucose in the liver (this confirms the results of

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153. Evans, G. Endocrinology. 29: 731. 1941

154.

Deuel et al¹⁵⁴), and (c) the curves indicate that the adrenalectomized rat with adrenocortical transplants does not store glycogen in the liver at a rate comparable to the intact animal, and that in a seven hour period it does not reach the levels attained by the normal animal.

Figure 9: (a) the adrenalectomized rat stores glycogen in the muscles but (b) not at the same rate as does the normal or the rat with transplants. (c) The normal rat and the rat with transplants keep pace with each other in their ability to store glycogen in the muscle for the first four hours after the glucose meal, but at this point the storage mechanisms of the rat with transplants appear to have settled down to a steady rate, lower than that of the normal, while the rate at which the normal rat stores glycogen continues to increase.

From these data, it is observed that differences appear to exist between the rat with transplants and the normal animal in three points, which are restated here: 1. a very slight difference in blood glucose tolerance; 2. a leveling off of the storage glycogen in the muscle of the 'transplant' after several hours (four); and 3. a failure of the 'transplant' to deposit glycogen in the liver in the same amounts as the normal animal. Explanations must be considered for these differences.

Using glycogen storage as a criterion of functional efficiency, the data demonstrate that the animal with transplants is not as efficient an organism as the normal animal in this respect. This impairment of function might be due to a difference in the quantity or quality of the secretion elaborated by the transplanted gland, and/or a medullary lack.

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154. Deuel, H.J., Jr., L.F. Hallman, S. Murray, and L.T. Samuels.
J. Biol. Chem. 119: 607. 1937

Since no specific data exists as to kind and amount of secretion, the cause of the difference can only be speculated upon.

First, could a lowered rate of absorption account for the lowered glycogen storage? In answering this question, the blood glucose tolerance offers some evidence against lowered absorption as a factor. Cori¹⁵⁵ has established the fact that in adrenalectomized animals, absorption of glucose is greatly retarded. But the weight of the evidence¹⁵⁶ suggests that this may be attributed to a disturbance of salt metabolism. Evans¹⁵⁷ has demonstrated that as far as return to original levels is concerned (after glucose injection) the adrenalectomized animal has a normal glucose tolerance. Figure 7 indicates that the blood glucose tolerance of the 'transplant' is normal, though the changes are not as abrupt nor the levels reached quite as high. While this deviation appears to be a minor one, further study may show it to be of greater significance. In line with this, however, it may be added that Wyman and tum Suden¹⁵⁸ presented evidence that the reaction to hyperglycemic-inducing agents, e.g. adrenaline, produces less of an elevation of the blood sugar in the 'transplant' than in the normal. This suggests an impairment in the 'transplant' of the mechanisms of carbohydrate metabolism. On the basis of the evidence presented here, it appears that no gross differences in the rate of absorption exist in the 'transplant'.

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155. Cori, C.F. *Physiol. Rev.* 11: 143. 1931

156, Clark, W.G. and E.M. MacKay. *Am. J. Physiol.* 137: 104. 1942

157. Evans, G. *Endocrinology.* 29: 731. 1941

158. Wyman, L.C. and C. tum Suden. *Am. J. Physiol.* 126: 7. 1939

Considering the curve of muscle glycogen storage next, how can a leveling off of the muscle glycogen content be accounted for? Could the explanation be an increased oxidation of carbohydrate? The curve of muscle glycogen (figure 9) represents at any point a balance between the amount of glycogen being deposited in the muscle tissues and the amount of glycogen being utilized by the muscle in its metabolic activities. As far as is known, the adrenalectomized rat shows no primary defect in its ability to oxidize carbohydrate (Ingle)¹⁵⁹. Corticosterone apparently decreases the proportion of glucose oxidized and increases its deposition (Long, Katzin, and Fry)¹⁶⁰. Thorn et al¹⁶¹ have shown an increased utilization of glucose in adrenalectomized animals. In the fasting adrenalectomized animal, these factors - poor formation but rapid use of glucose - would account for the rapid exhaustion of the glycogen stores. Britton¹⁶² has state that the adrenalectomized animal is unable to form liver and muscle glycogen from ingested carbohydrate to any noteworthy degree. Contrary to this, Long et al¹⁶⁰ found that so long as the adrenalectomized animal is fed, normal levels of glycogen are found. In the group of adrenalectomized animals used here, the animals showed a low muscle glycogen after fasting, but proceeded to deposit glycogen in the muscles after a glucose meal. This coincides with the findings of Long et al¹⁶⁰. The 'transplant' reacts in a different manner. The curve (figure 9) indicates that after ingestion of glucose,

159. Ingle, D.W. Endocrinology. 31: 419. 1942

160. Long, C.N.H., B.Katzin, and E.G.Fry. Endocrinology. 26: 309. 1940

161. Lewis, E.A., D.Kuhlman, C.Helbue, G.F.Koepf, and G.W.Thorn.
Endocrinology. 27: 971. 1940

162. Britton, S.W. Am. J. Physiol. 126: P443. 1939

the 'transplant' reacts like the normal for several hours, but then fails to store as much muscle glycogen as the normal animal. This could be due to a drop in the rate of glycogenesis, or to increased utilization. On the basis of experimentation on the adrenalectomized animal, it is reasonable to suspect that increased utilization explains this difference in the 'transplant', and that the animal with transplants is deficient in the amount (or kind) of hormone, produced by the regenerated cortical tissue, which controls glycogen storage in the muscle. This would appear to explain why the 'transplant' fails to keep pace with the normal animal after several hours.

Why is storage of liver glycogen decreased in the 'transplant'? The same line of reasoning can be followed here as for muscle glycogen. Two principle sources of glycogen are available to the liver: 1. from carbohydrate in the blood stream; 2. from deamination processes in the liver, giving rise to gluconeogenesis. The glycogen content at any moment, then, depends on the balance between glycogenesis from at least these two sources, and the glycogenolytic processes going on in the liver. The blood glucose has already been considered to be normal (in the 'transplant'), and is therefore excluded as a determining factor. Since the blood sugar level and adrenalin are the chief glycogenolytic agents, both of these may be excluded as factors responsible for the failure of glycogen storage (the adrenal medulla is lacking in the 'transplant'). Therefore it is reasonable to assume that very little glycogenolysis goes on in the liver after the glucose meal (at least not until the blood sugar level falls below the normal).

It has been shown (Long¹⁶³, Anderson¹⁶⁴) that adrenalectomized rats maintained on a high sodium intake are capable of depositing liver glycogen at a normal rate. This could reasonably be assumed to be true also of the 'transplant', and that glycogenesis proceeds in a normal manner. The factor of gluconeogenesis remains to be considered. In favor of this factor is the evidence that has been secured for the adrenalectomized rat. Long, Katzin, and Fry¹⁶⁵ demonstrated that the cortical hormones are essential for a normal rate of gluconeogenesis from protein. They observed that after administration of cortical extracts ". . . in fed animals very large quantities of glycogen accumulate in the liver and under these circumstances the muscle glycogen may also increase. . . ." Study of the metabolism of tissue slices taken from adrenalectomized animals has provided supporting evidence for this function of the cortical hormones. Long¹⁶⁶ has suggested that ". . . adrenalectomy retards the rate of deamination of amino acids and in consequence the rate of glucose formation from protein . . .". Thus a possible explanation of the decreased liver glycogen storage in the 'transplant' is a failure of the transplanted tissue to produce, to the same degree as the normal animal, the hormone controlling this factor.

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163. Long, C.N.H., B.Katzin, and E.G.Fry. Endocrinology 26: 309. 1940

164. Anderson, E., V.V.Herring, and M. Joseph.

Proc. Soc. Exper. Biol. Med. 45: 488. 1940

165. Long, C.N.H., B. Katzin, and E.G.Fry. Endocrinology. 26: 309. 1940

166. Long, C.N.H. Ann. Rev. Physiol. 4: 465. 1942

With respect to these phases of carbohydrate metabolism, the intermediacy of the adrenalectomized animal with cortical transplants to the normal, and adrenalectomized animal lends supporting evidence to the claim of Wyman and tum Suden¹⁶⁷ that ". . . transplanted cortical tissue does not function as efficiently as does the normal gland in acute emergencies. . . .".

Is impairment of the carbohydrate metabolism the explanation for the increased susceptibility of the transplant to histamine and anaphylactic shock? Is cortical control the whole answer or is medullary support necessary? C. tum Suden and Wyman¹⁶⁸ believe both to be important, though ascribing a lesser effect to the secretion of the medulla. As early as 1931, Cori¹⁶⁹ suggested that possibly the primary effect of adrenaline is to accelerate glycogenolysis in all cells. At the same time, he presented evidence which demonstrated that after the injection of adrenaline in rats in the fasting state, glycogen disappears from the muscle tissue, and accumulates in the liver. This he regarded as a secondary effect of the general glycogenolysis. Raab¹⁷⁰ has reported the presence of considerable amounts of adrenal cortical compounds (steroids) tied together with adrenaline "in the parenchymatous organs of the rat and to a lesser extent also in the muscles of the extremities where a comparatively large amount of 'free' adrenaline was found instead."

167. Wyman, L.C. and C. tum Suden. Endocrinology. 21: 587. 1937

168. tum Suden, C. and L.C. Wyman. Personal communication.

169. Cori, C.F. Physiol. Rev. 11: 143. 1931

170. Raab, W. Endocrinology. 29: 126. 1941

It seems reasonable to suspect that the cortical steroids and adrenaline may exert a synergistic effect on the organism, and in times of stress particularly. With a lack of either, impairment of function might result. C. tum Suden and Wyman¹⁷¹ have suggested that the "cooperation of adequate cortical and medullary function in the intact gland furnishes a margin of safety greater than possible in the transplant". Possibly an explanation of histamine and anaphylactic shock might be obtained by duplicating their experiments, and by some method manipulating the carbohydrate metabolism so as to observe the effects of the deviations from the normal on the source of events in shock.

The data presented here give further evidence that the adrenalectomized rat with adrenal cortical transplants reacts like the normal rat in certain respects (carbohydrate levels after 24 hours of fasting), but does not function as efficiently as does the normal animal in acute emergencies (e.g. after a glucose meal).

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171. tum Suden, C. and L.C.Wyman. Personal communication.

IX. CONCLUSIONS AND SUMMARY

1. Evidence is presented that the adrenalectomized rat with autoplasmic adrenal cortical transplants does not vary significantly from the normal animal with respect to its blood sugar, liver glycogen, or muscle glycogen, at the end of a 24 hour period of fasting.

2. After a glucose meal, the blood tolerance of the rat with 'transplants' approximates that of the normal animal.

It is demonstrated however, under these conditions, that glycogen storage in the liver and muscle of rats with 'transplants' is impaired to some degree.

3. It is concluded that

a) the carbohydrate levels in the adrenalectomized rat with autoplasmic adrenal cortical transplants are like those of the normal rat after a 24 hour fasting period;

b) the rat with 'transplants' differs from the normal animal in those mechanisms responsible for the storage of glycogen in the tissues, being intermediate in this respect to the normal and completely adrenalectomized rat, but more like the former;

c) possible explanations for this difference between the storage levels of the 'transplant' and the normal rat are that a relative cortical and/or medullary deficiency in the former leads to decreased gluconeogenesis in the liver and eventually, increased utilization of carbohydrate in muscle.

IX. DISCUSSION AND SUMMARY

1. Evidence is presented that the adrenomedullary rat with autophagic adrenal cortical transplants does not vary significantly from the normal animal in response to increased sugar, liver glycogen, or muscle glycogen, at the end of a 24 hour period of fasting.
2. After a glucose meal, the blood glucose of the rat with 'transplants' approximates that of the normal animal. It is demonstrated, however, under these conditions, that glycogen storage in the liver and muscle of rats with 'transplants' is impaired to some degree.
3. It is concluded that:
 - a) the carbonhydrate levels in the adrenomedullary rat with autophagic adrenal cortical transplants are like those of the normal rat after a 24 hour fasting period;
 - b) the rat with 'transplants' differs from the normal animal in that mechanism responsible for the release of glycogen in the tissues, being inadequate in this respect to the normal and possibly adrenomedullary rat, but more like the former;
 - c) possible explanation for this difference between the storage levels of the 'transplant' and the normal rat may be a relative cortical and/or medullary deficiency in the former leads to decreased gluconeogenesis in the liver and eventually, increased utilization of carbohydrate in muscle.

X. APPENDIXA) PREPARATION OF GLUCOSE SOLUTION FOR FEEDING PURPOSES

This solution was prepared according to the method of Cori¹⁷², who showed that the average amount of glucose absorbed in four hours by 24 hour fasting rats equaled 1.065 grams of glucose per 100 grams of body weight.

A given amount of chemically pure dextrose was weighed out (e.g. 25 grams). This was then dissolved in distilled water and made up to such a volume that 1 cc. contained 1 gram of glucose. This permitted quick computation of the number of cc. of solution to be fed to the animal, and also kept the volume small enough so as not to overload the stomach.

The solution was made up fresh for each day's feeding.

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172. Cori, C.F. and G.T. Cori. J. Biol. Chem. 79: 321. 1928

A) PREPARATION OF GLUCOSE SOLUTION FOR FEEDING PURPOSES

This solution was prepared according to the method of Gortliff, who showed that the average amount of glucose absorbed in four hours by 24 hour fasting rats was about 1.05 grams of glucose per 100 grams of body weight.

A given amount of anhydrous pure glucose was weighed out (e.g. 25 grams). This was then dissolved in distilled water and made up to such a volume that 1 cc. contained 1 gram of glucose. This permitted quick comparison of the number of cc. of solution to be fed to the animal, and also kept the volume small enough so as not to overload the stomach.

The solution was made up fresh for each day's feeding.

B) DETERMINATION OF GLYCOGEN^{173, 174}

The tissue (liver or muscle) to be analyzed was weighed immediately on removal and placed in a 25 x 225 mm. pyrex tube. 30 % potassium hydroxide was then added (approximately 2 cc. per gram of tissue) and the tube, unstoppered, was placed on a boiling water bath and heated until the tissue was completely in solution (a matter of 10-20 minutes). The glycogen was then precipitated with 1.1 to 1.2 volumes of 95 % alcohol. The whole was then heated again until the mixture began to boil. This heating period aided in the precipitation of the glycogen and caused it to adhere better to the walls of the tube. It was then cooled and centrifuged. The supernatant fluid was poured off, with no loss of precipitate. The glycogen was then dissolved in water, and reprecipitated with alcohol as just described.

For hydrolysis of the glycogen, normal sulfuric acid was added to the precipitated material and made up to a given volume. The whole then was heated in a boiling water bath for a period of at least three and one-half hours. (Various periods have been given for this period of heating, but a minimum period of three hours seems to be agreed upon). In general, the determination here made involved a hydrolysis period of 3 and 1/2 to 4 and 1/2 hours. The test-tube was provided with an air reflux condenser during the hydrolysis.

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173. Cori, C.F. and G.T. Cori. J. Biol. Chem. 100: 323. 1933

174. Good, C.A., H. Kramer, and M. Somogyi. J. Biol. Chem. 100: 485. 1933

2) DETERMINATION OF GLYCEROL

The tissue (liver or muscle) to be analyzed was weighed immediately after removal and placed in a 250 cc. Pyrex tube. 30 cc. potassium hydroxide was then added (approximately 5 cc. per gram of tissue) and the tube, unstoppered, was placed on a boiling water bath and heated until the tissue was completely dissolved (a matter of 10-20 minutes). The glycerol was then precipitated with 1.1 to 1.2 volumes of 95% alcohol. The whole was then heated again until the mixture began to boil. This heating period also is the precipitation of the glycerol and causes it to adhere better to the walls of the tube. It was then cooled and centrifuged. The supernatant fluid was poured off, with no loss of precipitate. The glycerol was then dissolved in water, and reprecipitated with alcohol as just described.

For hydrolysis of the glycerol, normal sulfuric acid was added to the precipitated material and made up to a given volume. The whole then was heated in a boiling water bath for a period of at least three and one-half hours. (Various periods have been given for this period of heating, but a minimum period of three hours seems to be agreed upon). In general, the determination here made involves a hydrolysis period of 3 and 1/2 to 4 and 1/2 hours. The test-tube was provided with an air return condenser during the hydrolysis.

173. Gott, C.F. and E.F. Dool. J. Biol. Chem. 1931: 252, 1932
174. Joss, C.A., H. Kress, and H. Rosenberg. J. Biol. Chem. 1933: 485, 1933

When hydrolysis was complete, an aliquot was neutralized with sodium hydroxide (using phenol red as an indicator) and made up to a given volume.

To 5 cc. of the neutralized hydrolysate, 5 cc. of the Shaffer-Hartman reagent was added, and the glucose content determined.

To convert the glucose thus determined to glycogen, the factor 0.927 was employed.

When hydrolysis was completed, an aliquot was neutralized with
sodium hydroxide (which showed two as an indicator) and made up to a
given volume.

To 5 cc. of the neutralized hydrolyzate, 5 cc. of the standard-
Hartman reagent was added, and the glucose content determined.
To compare the glucose thus determined to glucose, the
factor 0.927 was required.

C) DETERMINATION OF GLUCOSE¹⁷⁵

The principle of this method is essentially as follows: the sugar solution is boiled with an alkaline copper solution. The residual cupric salt is then converted into cuprous iodide with the liberation of an equivalent amount of iodine. The iodine liberated is then titrated with standard sodium thiosulfate and the sugar values obtained from a chart (made on known sugar solutions).

5 cc. of the sugar solution were measured into a Pyrex test-tube, followed by 5 cc. of the copper reagent, added in a manner to rinse the sugar solution from the walls of the tube. The solutions were mixed with gentle shaking and the tubes covered with tin-foil. A blank was always run with any determination, using five cc. of water in place of the sugar solution. The tubes were then placed in a vigorously boiling water bath for a period of 15 minutes. (The period of boiling was exactly the same as the period for which the chart for known sugar solutions was made out). At the end of the period, the tubes were removed to cold water and allowed to cool to about 30°. When cooled, the solutions were acidified with N sulfuric acid (5 cc.) and gently mixed. (Vigorous mixing may permit a certain amount of re-oxidation and lead to erroneous results). The solutions were permitted to stand (covered) until complete dissolution of the cuprous oxide had come about. The whole was then transferred quantitatively to a 250 cc. Erlenmeyer flask, and titrated with 0.005 N thiosulfate, adding 1 cc. of 1% starch solution

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175. Shaffer, P.A. and M. Somogyi . J. Biol. Chem. 100: 695. 1933

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DETERMINATION OF SUGAR

The principle of this method is essentially as follows: the sugar solution is boiled with an alkaline copper solution. The residual cuprous salt is then converted into cuprous iodide with the liberation of an equivalent amount of iodine. The iodine liberated is then titrated with standard sodium thiosulfate and the sugar values obtained from a chart (made on known sugar solutions).

2 cc. of the sugar solution were measured into a 15-ml. test-tube, followed by 2 cc. of the copper reagent, added in a manner to rinse the sugar solution from the walls of the tube. The solution was stirred with gentle shaking and the tube covered with tin-foil. A blank was always run with any determination, using 15 cc. of water in place of the sugar solution. The tubes were then placed in a vigorously boiling water bath for a period of 15 minutes. (The period of boiling was exactly the same at the period for which the chart for known sugar solutions was made out). At the end of the period, the tubes were removed to cold water and allowed to cool to about 30°. When cooled, the solutions were acidified with N sulfuric acid (5 cc.) and gently mixed. (Vigorous mixing may permit a certain amount of re-oxidation and lead to erroneous results). The solutions were permitted to stand (covered) until complete dissolution of the cuprous oxide had come about. The whole was then transferred quantitatively to a 150 cc. Erlenmeyer flask, and titrated with 0.005 N thiosulfate, adding 1 cc. of 1% starch solution

175. Shallen, F. A. and W. R. Murphy. J. Biol. Chem. 100: 695, 1935

toward the end-point. The titration value was subtracted from the heated blank titration value, and the titration difference referred to a previously worked-out chart from which were obtained the values of glucose. (See figure 10).

Composition of the copper-iodometric reagent

Na_2CO_3 (anhydrous)	25	gms.	per	liter
NaHCO_3	20	"	"	"
Rochelle salt	25	"	"	"
$\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$	7.5	"	"	"
KIO_3 , 0.1 N as to I_2	.100	cc.	"	"
KI	5	gms.	"	"

GLUCOSE (IN MGMS)

FIG 10. CURVE FOR DETERMINATION OF GLUCOSE
 METHOD OF CHAPMAN & SMOGGIN
 USING REAGENT 20, WITH A GARD 41

oward the end-point. The titration value was subtracted from the heated blank titration value, and the titration difference referred to a previously worked-out chart from which were obtained the values of glucose. (See figure 10).

Composition of the copper-toluenesulfonate reagent

Na_2CO_3 (anhydrous)	25 gms. per liter
NaHCO_3	30 "
Sodium salt	25 "
$\text{CaSO}_4 \cdot 2\text{H}_2\text{O}$	7.5 "
KIO_3 , 0.1 N as I_2	100 cc.
KI	5 gms.

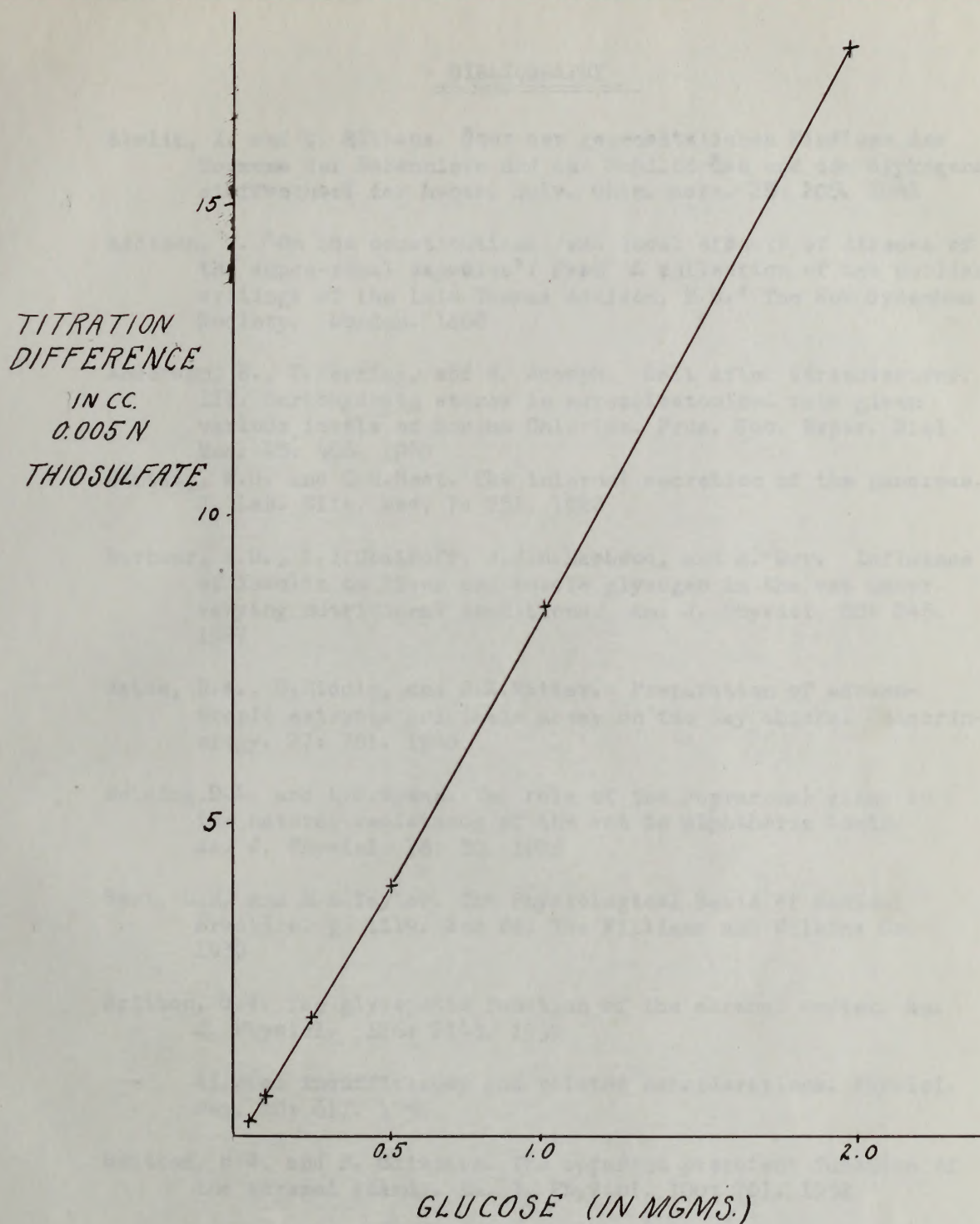


FIG. 10. CURVE FOR DETERMINATION OF GLUCOSE
METHOD OF SHAFFER & SOMOGYI
USING REAGENT 50, WITH 5 GMS. KI

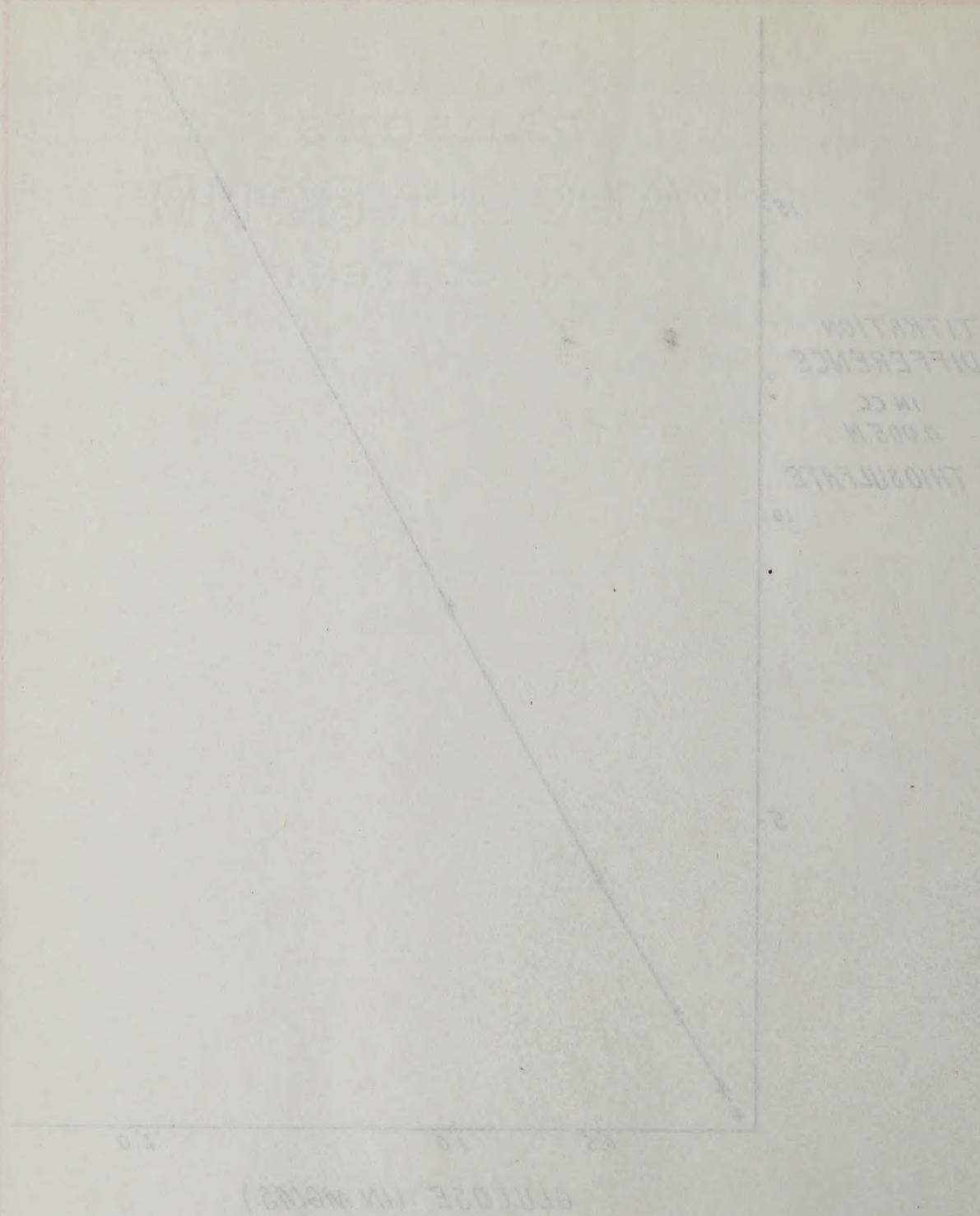


FIG. 10. CURVE FOR DETERMINATION OF GLUCOSE
 METHOD OF SHAFER & SOMMER
 USING REAGENT 50, WITH 5 mg. KI

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CARBOHYDRATE METABOLISM IN RATS WITH ADRENOCORTICAL TRANSPLANTS

Abstract of a Dissertation

Submitted in partial fulfilment of the requirements for the degree of

Doctor of Philosophy

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CARBOHYDRATE METABOLISM IN RATS WITH ADRENOCORTICAL TRANSPLANTS

Many studies have been made of the part played by the adrenal cortex in carbohydrate metabolism. The conclusions of these studies are chiefly based on the use of animals in the terminal stages of adrenal insufficiency, and are therefore subject to criticism. The use of the adrenalectomized rat with autoplasmic adrenocortical tissue is considered to obviate some of these criticisms.

A review of the literature of carbohydrate metabolism and the factors affecting it is given. The regulation of carbohydrate metabolism by the glands of internal secretion is discussed, with emphasis on the part played by the adrenal cortex. Adrenal cortical hormones possessing "cortin activity", and their assay, are reviewed, their comparative effects considered and summarized. A review of the studies made on carbohydrate metabolism in adrenalectomized rats is included.

The problem of relative insufficiency of adrenocortical hormone is discussed and the literature reviewed. Studies of the rat with transplanted cortical tissue have shown that the transplanted tissue is able to maintain the life and health of the animal under ordinary conditions, but is not able to function as efficiently as does the normal gland under conditions of stress. The blood sugar in the rat with transplanted cortical tissue has been investigated but the literature contains no studies of the glycogen levels in such animals.

Many studies have been made of the part played by the animal cortex in memory, especially the organization of these studies are chiefly based on the use of animals in the laboratory of animal psychology, and are therefore subject to criticism. The use of the experimentalist has with increasing experimental design is considered to be a source of error.

A review of the literature of comparative psychology and the factors affecting it is given. The organization of comparative psychology by the animal is discussed, and the organization of the part played by the animal cortex in memory is discussed. The organization of memory, and the organization of memory, are discussed. A review of the studies made on comparative psychology, in experimental design is given.

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EXPERIMENTAL PART. A mixed stock of male albino rats were bilaterally adrenalectomized (by the lumbar approach), followed by implantation of the removed glands (all in one operation) and a sufficient period of recovery (not less than six weeks). From these, one group of rats was subjected to a fasting period of 24 hours. Free access to water was allowed during the period. At the end of the fasting period, the rats were anesthetized with sodium amytal (intraperitoneal injection - 10 mgms. per 100 gms. body weight). The abdominal cavity was opened and a sample of blood was obtained from the vena cava. The whole liver then was removed, weighed, and immediately placed in hot 30% potassium hydroxide solution. Samples of gastrocnemius muscle were obtained, weighed, and promptly placed in hot 30% potassium hydroxide. Determinations were made of the blood glucose (using the Somogyi-Shaffer-Hartman iodometric titration method), and of the glycogen of the liver and muscles (using Good, Kramer, and Somogyi's modification of Pflüger's method). A control group of intact animals was subjected to the same procedure.

On other groups (including one group of completely adrenalectomized rats) a glucose meal was given at the end of the 24 hour fasting period. The procedure was as follows: At the end of the 24 hour fasting period, the rats were fed glucose solution (1.063 gms. glucose per 100 gms. body weight) by stomach tube without anesthesia or excitement. After an interval of one hour, one animal from each group was anesthetized. Samples of blood and gastrocnemius muscle, and the whole livers were removed for glucose or glycogen analyses. The same procedure

was carried out at the end of two, three, and so on, hours, up to seven hours, after the glucose meal. Determinations were made of the blood glucose, and the glycogen of the liver and muscles.

RESULTS. At the end of the 24 hour fasting period, in seven normal male rats the blood glucose averaged 77 mgms. per 100 cc. whole blood (range, 66 - 93 mgms.); liver glycogen, 32 mgms. per 100 gms. wet liver (range, 8 - 83 mgms.); and muscle glycogen, 323 mgms. per 100 gms. wet muscle (range, 240 - 433 mgms.).

Eleven male rats with adrenocortical transplants showed the following picture after 24 hours of fasting: blood glucose averaged 80 mgms. per 100 cc. whole blood (range, 62 - 94 mgms.); liver glycogen 35 mgms. per 100 gms. wet liver (range, 4 - 91 mgms.); and muscle glycogen, 484 mgms. per 100 gms. wet muscle (range, 400 - 591 mgms.).

These values are considered to be within the normal range, and in agreement with average values obtained by other investigators. It is concluded that the adrenalectomized rat with transplanted adrenal cortical tissue is like the normal rat after a 24 hour fasting period with respect to blood glucose, liver glycogen, and muscle glycogen.

In the groups of animals given a glucose meal, the following results were obtained. The glucose tolerance curve of the rat with adrenocortical transplants was like that of the normal with respect to shape, but the curve was less sharp, and had slightly lower values than that of the normal intact animal. With respect to liver glycogen, the adrenalectomized animal failed to deposit any during a five hour period; the adrenalectomized rat with adrenocortical transplants did

not store glycogen in the liver at a rate comparable to the intact animal, and in a seven hour period did not reach the levels attained by the normal animal. The adrenalectomized rat stored glycogen in the muscles, but not at the same rate as the normal or the 'transplant'. The normal rat and the rat with 'transplants' kept pace with each other in their ability to store glycogen in the muscles for the first four hours after the glucose meal, but at that point the storage mechanisms of the rat with 'transplants' appeared to have settled down to a steady rate, lower than that of the normal, while the rate at which the normal rat stored glycogen continued to increase.

On the basis of these results, the glucose tolerance curve in the rat with 'transplants' is considered to be normal, and it is concluded that no gross differences in the rate of absorption exist in the 'transplant'.

Explanations are considered for the leveling off of the curve of glycogen storage in the muscle of the 'transplant'. On the basis of previous evidence obtained on the adrenalectomized rat, it is suspected that increased utilization explains this difference between the 'transplant' and the normal, and that the animal with 'transplants' is deficient in the amount (or kind) of hormone produced by the regenerated cortical tissue which controls glycogen storage in the muscle.

The impaired ability of the rat with 'transplants' to store glycogen in the liver at the same rate or to the same extent as the normal is discussed. It is possible that in the 'transplant' there is a failure of the transplanted tissue to produce, to the same degree as

not acute hypoxia in the liver of a rat comparable to the normal animal, and in a given hour period the rate of oxygen consumption by the normal animal. The above-mentioned rat, which was killed by the normal animal, but not at the same rate as the normal animal. The normal rat and the rat with 'transformation' were kept with each other in their ability to store oxygen in the tissues for the first hour after the hypoxic test. The rat with the 'transformation' of the hypoxic test, which was killed at a rate which was the same as the normal rat, was the rate at which the normal rat stored oxygen in its tissues. On the basis of these results, the hypoxic test is considered to be normal, and it is concluded that no great difference in the rate of oxygen consumption in the 'transformation'.

Experiments are conducted for the leveling off of the rate of oxygen storage in the tissues of the 'transformation'. On the basis of previous evidence obtained on the 'transformation', it is concluded that the hypoxic test is a difference between the 'transformation' and the normal rat, and the animal with 'transformation' is killed in the second (or high) of hypoxic period in the hypoxic test. The hypoxic test is a hypoxic test in the normal rat.

The hypoxic test of the rat with 'transformation' is stored oxygen in the liver of the rat or for the first hour in the normal is discussed. It is possible that in the 'transformation' there is a failure of the hypoxic test to proceed in the same manner as

the normal, the hormone which controls gluconeogenesis in the liver.

It is suggested that an impairment of the carbohydrate metabolism may be related to the increased susceptibility of the 'transplant' to histamine and anaphylactic shock. It is also suggested that the hormones of the cortex may act synergistically with adrenaline on the organism in times of stress particularly.

The intermediacy of the 'transplant' to the normal and the adrenalectomized rat with respect to carbohydrate metabolism is considered, and it is observed that the evidence supports the theory that the adrenalectomized rat with transplanted cortical tissue does not function as efficiently as does the normal animal in acute emergencies.

It is concluded that (a) the carbohydrate levels in the adrenalectomized rat with adrenocortical transplants are like those of the normal rat after a 24 hour period of fasting; (b) the rat with 'transplants' differs from the normal animal in those mechanisms responsible for the storage of glycogen in the tissues, being intermediate in this respect to the normal and the completely adrenalectomized rat, but more like the former; (c) possible explanations for this difference between the storage levels of the 'transplant' and the normal rat are that a relative cortical and/or medullary deficiency in the former leads to a decreased gluconeogenesis in the liver, and eventually increased utilization of carbohydrate in the muscle.

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